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May 2004

Contingency Plan for Hawaiian Monk Seal Unusual Mortality Events

Pamela K. Yochem, Robert C. Braun, Bradley Ryon,
Jason D. Baker, and George A. Antonelis

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Pacific Islands Fisheries Science Center
National Marine Fisheries Service
National Oceanic and Atmospheric Administration
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Contingency Plan for Hawaiian Monk Seal Unusual Mortality Events

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NOAA Technical Memorandum NMFS-PIFSC-2

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CONTENTS

Contents	Page
1. Background	1
1.1 Distribution and Habitat Use	1
1.2 Population Biology	1
1.3 Mortality Patterns	3
1.4 Unusual Mortality: Past Events	5
1.4.1 Hawaiian Monk Seal Unusual Mortality Event, Laysan Island, 1978	5
1.4.2 Hawaiian Monk Seal Unusual Mortality Event, Laysan Island, 2000-2001	5
1.5 Background of this Document	6
2. Federal/State Authority and Jurisdiction	6
3. The On-Site Coordinator	7
3.1 The Appointment of the On-Site Coordinator	7
3.2 Responsibilities of the Federal On-Site Coordinator	8
4. Pre-Event Planning for Rapid Response	8
4.1 Building a Response Team	8
4.2 The Administrative Support Team	9
4.3 Scientific Advisors	9
4.4 Training and Readiness	9
4.5 Establishing MOUs with Cooperators	10
4.6 Pre-Planning for Sample Analyses	10
5. Recognizing an Unusual Event	10
5.1 Guidelines for a Graduated Response	11
5.2 Protocol for Initiating Unusual Event Designation	12
6. Notification of Personnel and Agencies	12
7. A Tailored Response	12
7.1 Objectives and General Guidelines	12
7.2 Responding to an Event of Known Cause	13
7.3 Investigating an Event of Unknown Cause	14
7.4 Determining "The End"	14
7.5 Post-Event Assessment and Monitoring	15
8. Communication and Reporting	15
9. Public Health Concerns	15

(**CONTENTS**, continued)

	Page
10. Resources: Location and Utilization	16
10.1 Introduction	16
10.2 Equipment and Logistics	16
10.3 Information and Data Resources	16
10.4 Development of Temporary Animal Housing and Emergency Facilities	17
10.5 Funding	17
11. Response and Investigation Protocols	17
11.1 Rescue and Rehabilitation	17
11.2 Release of Rehabilitated Monk Seals after a MMUME	17
11.3 Procedures for Carcasses	17
11.4 Tissue Sample Collection	17
11.5 Environmental Assessment and Sampling	18
11.6 Packaging and Shipping Samples	18
11.7 Keeping Records and Maintaining Chain of Custody	18
11.8 Surveys	18
12. Acknowledgments	18
13. References	19
14. List of Appendices	23
A: Hawaiian Monk Seal Specimen Collection	A-1
B: Hawaiian Monk Seal Necropsy Protocol	B-1
C: Equipment and Supplies for a Single Necropsy	C-1
D: Hawaiian Monk Seal Rescue and Rehabilitation Guidelines	D-1
E-1: Additional Samples Requested during the Laysan 2001 MMUME	E-1
E-2: Specimen Collection Protocols for Specific Biotoxins	E-3
E-3: Specimen Collection Protocols for Chemical Pollutants	E-7
F-1: Epidemiology Sampling Supply List	F-1
F-2: Veterinary Medical Kit	F-5
G-1: Monk Seal Specimen Collection Summary Form	G-1
G-2: Hawaiian Monk Seal Tagging & Chemical Immobilization Form	G-3
G-3: Hawaiian Monk Seal Clinical Examination Form	G-7
G-4: Hawaiian Monk Seal Field Hematology Report	G-11
G-5: NMFS Human Interaction Forms	G-15
G-6: Marine Forensics Chain of Custody Form	G-19
H-1: Federal, State and Local Authorities	H-1
H-2: Potential Response Team Members	H-3
H-3: Diagnostic Laboratories	H-7
H-4: Cooperating Experts	H-11

(**CONTENTS**, continued)

	Page
I-1: Flowchart and timing of response to MMUME in the United States	I-1
I-2: Flow Diagram for Suspected Marine Biotoxin Incidents	I-3
I-3: Flowchart for response to a marine mammal mortality event with known vs. unknown cause	I-7
J-1: MOU with participants in UME response	J-1
J-2: MOU with laboratories/individuals performing tests/analyses	J-3
K-1: Excerpts, 28 August 2001 Update of the Hawaii Area Contingency Plan	K-1
K-2: Excerpts, Oil Spill Field Operations Guide	K-5

1. Background

1.1 Distribution and Habitat Use

Hawaiian monk seals (*Monachus schauinslandi*) were designated as depleted under the Marine Mammal Protection Act and endangered under the Endangered Species Act in 1976, following declines of 50% from the late 1950s. In 1988, critical habitat for monk seals was designated as the emergent land, lagoon waters, and ocean waters out to a depth of 20 fathoms (37 m) around breeding islands (excluding the main Hawaiian Islands) and Maro Reef. In 1991, a Protected Species Zone was established out to 50 nautical miles from the islands and the corridors between islands.

There are six main reproductive populations of Hawaiian monk seals in the Northwestern Hawaiian Islands (NWHI): French Frigate Shoals (FFS), Laysan Island, Lisianski Island, Pearl and Hermes Reef, Midway Atoll, and Kure Atoll (Fig. 1). Smaller numbers are present on Necker Island and Nihoa Island and on the main Hawaiian Islands. Monk seals are rarely observed at Johnston Atoll where one female pup was born in 1969.

Approximately 90% of Hawaiian monk seals remain at their natal site for life; the remaining 10% move between or among major population centers. Studies conducted at FFS in 1996 and 1997 documented the use of foraging habitats by monk seals which were much farther from breeding locations than was previously known or anticipated. Similar studies in 1997-98 at Pearl and Hermes Reef documented very little extra-atoll movement. Most feeding appears to occur at depths less than 75-90 m, though seals occasionally dive to depths exceeding 500 m. Known prey items include reef fishes, benthic fishes, cephalopods, and crustaceans.

1.2 Population Biology

Since 1985, the average rate of decline was approximately 3% yr^{-1}, although the beach counts[1] have been stable from 1993 to 2000 and declined again in 2001 and 2002 (NMFS, unpublished data). Further decline is likely due to high juvenile mortality and an inverted age structure at FFS, the largest colony (Carretta et al., 2001). The annual number of births has varied substantially over the past decade and is expected to decline in the near future due to poor recruitment at FFS. At present the species numbers approximately 1400 and is the most endangered species of marine mammal that lives entirely within U.S. jurisdiction (Carretta et al., 2002).

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[1] Direct enumeration data cannot be used for characterizing long-term trends because sufficient field investigation in the NWHI has not been consistently undertaken at all sites and years. Instead, a measure of long-term trend is derived from the mean of all the beach counts that have been conducted with varying frequency since the late 1950s. Beach counts provide a useful index of population trends and do not account for seals at sea. Approximately 1/3 of each seal population is on land during a survey, but the exact proportion varies (e.g., by site and season).

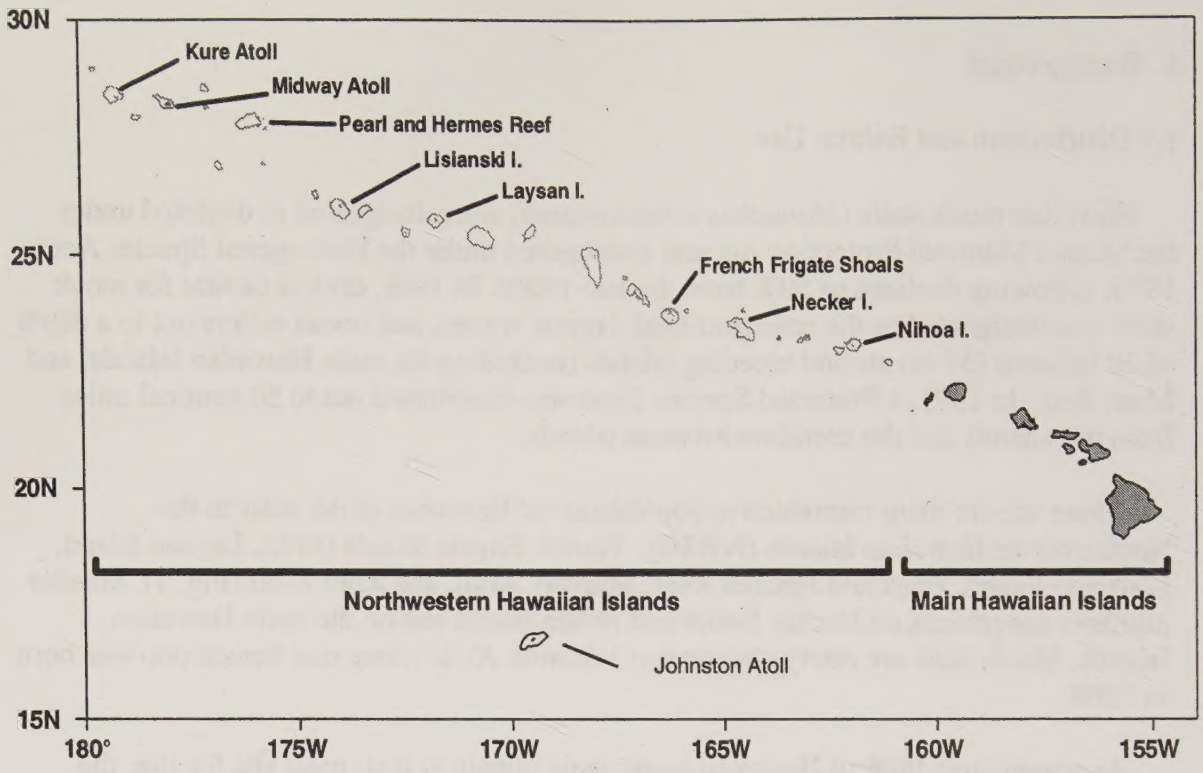

Fig. 1. The Hawaiian Archipelago, with the six main monk seal breeding and pupping sites identified (French Frigate Shoals, Laysan I., Lisianski I., Pearl and Hermes Reef, Midway Atoll, Kure Atoll).

Females give birth on beaches with adjoining shallow waters, which provide protection from sharks. Most pups are born between February and August, with a peak from late March to May (Johnson and Johnson, 1980; Johanos et al., 1994). Females give birth for the first time at ages 5 to 10, and typically 50%-70% of all adult-size females give birth each year (Johanos et al., 1994). Gestation is approximately 1 year, with a mean interval between births (for those females that pup in consecutive years) of 381 days (Johanos et al., 1994). Females fast during the 5 or 6-week nursing period, and nursing pups are sometimes exchanged between females (Kenyon and Rice, 1959; Alcorn and Henderson, 1984; Boness, 1990; Gerrodette et al., 1992). Mating occurs 3 or 4 weeks after pups are weaned. Although mating takes place in the water and is rarely observed, a mating peak in May and June is inferred from the occurrence of fresh mounting injuries and association patterns of males and females (Johanos et al., 1994). Unlike other gregarious pinnipeds, monk seals are typically solitary and do not form large, seasonal breeding aggregations. They have a serially monogamous social system with males forming a dominance hierarchy.

Pups are born with a black coat, which is shed gradually at the end of the nursing period and replaced by pelage that is silver gray on the dorsum and sides and beige on the venter. Juveniles and adults undergo a "catastrophic" annual molt, in which the epidermis

is shed with the hair in large patches (Kenyon and Rice, 1959; Yochem and Stewart, 2001). The freshly molted pelage is silver gray above and beige below except in adult males, where it is dark brown. Molting peaks in July, and females with pups molt 5 or 6 weeks after mating (Johanos et al., 1994).

1.3 Mortality Patterns

Compared to other marine mammals, very good information exists on mortality rates of Hawaiian monk seals. These rates are obtained annually by resighting known-age animals during 2-5-month field seasons at all six main subpopulations in the NWHI (Fig. 1). Current tag and resight efforts are sufficient to obtain cohort-and age-specific survival (the complement of mortality) rates using Jolly-Seber analysis. These data are analyzed annually, and estimated rates are back-corrected when animals not seen for one or more years are subsequently resighted. Thus, long-term trends and variability in monk seal mortality rates are well characterized to age 15 years or more. These rates have reflected trends that greatly influence monk seal population trends. For example, juvenile mortality rates were low at most sites during the mid-to-late 1980s. Subsequently, juvenile mortality increased throughout the archipelago to varying degrees and on different time frames. Most notably, very high juvenile mortality has led to a severe and enduring population decline at FFS. Recent decline in juvenile survival has also been reported at Pearl and Hermes Reef and Midway and Kure Atolls in 2001 and 2002 (NMFS unpublished data).

Although annual mortality rates are well characterized, the timing and relative importance of various causes of mortality are not well known. Jolly-Seber rates are available only at the end of a field season, so these data cannot be used to evaluate mortality processes in real time. Moreover, as noted above, these rates are annually updated and back-corrected with each new year's data. The exact timing of mortalities is rarely known, as field effort encompasses less than half of any given calendar year. Even within a field season it is rarely possible to determine when an animal has died because so few carcasses are observed on the beach. In the few cases where carcasses are available for examination, unequivocal cause of death is often not discernible. For animals that are no longer sighted during regular beach walks, one can only infer that they died some time after the final sighting. For the Jolly-Seber analysis, any animal sighted within the calendar year, whether the first day or the last, is scored as having survived to that year. This is necessary for calculation of annual rates but does not provide within-season information on time of death.

To address the information gaps noted above, field teams from the Hawaiian Monk Seal Assessment Program provide a weekly report of the cumulative number of 1- to 3-year-old seals sighted to date, as well as the total number of confirmed and probable deaths within the season. In this way, minimum juvenile survival is regularly assessed so that unusually high mortality within a field season is likely to be detected. Post-mortem exams are performed, and samples are collected for evaluation by a team of marine mammal health and disease experts to determine healthy body condition and cause of death where possible. Some laboratory analyses (e.g., erythrogram and leukogram evaluations) are performed in the field; these preliminary findings, as well as descriptions

of gross lesions or clinical signs observed, are reported to the Monk Seal Assessment Program and Monk Seal Health and Disease Program. These measures increase the likelihood of timely detection of an unusual mortality event, allowing a response to be mounted within the field season if deemed appropriate.

Known causes of mortality in Hawaiian monk seals include emaciation of juveniles, tiger and Galapagos shark attacks, male aggression (individual and multiple), deleterious fisheries interactions, and entanglement in marine debris (Balazs and Whittow, 1979; Kenyon, 1981; Henderson, 1985, 1990; Alcorn and Kam, 1986; Banish and Gilmartin, 1992; Hiruki et al., 1993a, 1993b; Nitta and Henderson, 1993; Starfield et al., 1995). Disturbance of pregnant or nursing females likely causes them to desert preferred pupping beaches, resulting in decreased pup survival (Kenyon, 1972; Kenyon, 1981). Maximum age is believed to be 25-30 years, but few seals live this long (NMFS, unpublished data).

The influence of disease on monk seal populations is not well understood, but is an active area of research (Aguirre et al., 1999; Aguirre, 2000; Appendix A, Hawaiian Monk Seal Specimen Collection Protocol and Appendix B, Hawaiian Monk Seal Necropsy Protocol). Infectious and noninfectious diseases have been reported in Hawaiian monk seals but do not appear to be impeding population recovery: all wild seals carry parasites; dental and skeletal abscesses and pathology were the apparent causes of death in one aged seal; and biotoxins may pose a serious risk (Golvan, 1959; Rausch, 1969; Kenyon and Rauzon, 1977 [cited in Kenyon 1981]; DeLong, 1978; DeLong and Gilmartin, 1979; Gilmartin et al., 1980; Whittow et al., 1980; Dailey et al., 1988). Of the 15 Marine Mammal Unusual Mortality Events (UMEs) occurring in other marine mammals species since 1992 (Dierauf and Gulland, 2001: 72-73, Table 1), infectious diseases and biotoxins were the most common diagnoses (five cases each). Other factors implicated in UMEs included fisheries interactions, ship strikes, and large-scale decadal changes in oceanic productivity (Polovina et al., 1995).

In a recent review of emerging and resurging diseases in marine mammals, Miller et al. (2001) describes three ways in which wildlife species may be exposed to emerging diseases (newly evolved or newly identified as pathogens), all three of which are applicable to Hawaiian monk seals. The first is exposure via spillover from domestic species or humans. Many recent reports of transmissible diseases in marine mammals have included organisms traditionally associated with domestic animals and humans (e.g.; *Brucella* sp., Ewalte et al., 1994; Ross et al., 1994; Jepson et al., 1994; *Mycobacterium bovis* and *M. tuberculosis*, Forshaw and Phelps, 1991; Cousins et al., 1993; Woods et al.; 1995; Bernardelli et al., 1996). The second method of exposure is via rehabilitation or translocation of animals (e.g., Stamper et al., 1998, reports the possible spread of leptospirosis from skunks to rehabilitating harbor seals). The third way that wildlife species may become exposed to emerging diseases is through the effects of environmental phenomena such as El Niño and large-scale climate change on the proliferation or spread of infectious organisms (Fauquier et al. 1998; Harvell et al., 1999; Reddy et al. 2001). Another source of exposure to infectious diseases in Hawaiian monk seals is other wildlife species. Morbillivirus has been detected in the North Pacific Ocean

(in common dolphins; Reidarson et al, 1998), and cross-species infection has been described for this group of viruses (e.g., canine distemper in Baikal and Caspian seals; Mamaev et al., 1995; Forsyth et al., 1998). A virus closely related to dolphin morbillivirus was implicated in an UME involving Mediterranean monk seals (Osterhaus et al., 1998), although saxitoxins were also detected in these seals.

1.4 Unusual Mortality: Past Events

1.4.1 Hawaiian Monk Seal UME, Laysan Island, 1978.

At least 50 monk seals died at Laysan Island in 1978, primarily in juvenile-young adult (1-5 years) and older adult (18-30 years) age classes (Johnson and Johnson, 1981; Gilmartin et al., 1980). Clinical signs noted before death included gradual weight loss (over a period of weeks to months), lethargy, change in behavior patterns, poor quality pelage (particularly around the face and neck), and presence of fresh lesions (primarily on the rear flippers and lower back). Affected seals were emaciated and gastric ulceration was observed in all 15 dead seals for which complete tissue sets were collected; this was accompanied by frank hemorrhage secondary to heavy gastric nematode infestations in many seals. High levels of ciguatoxin and maitotoxin, neurotoxins produced by benthic dinoflagellates in association with tropical reef environments, were estimated by bioassay analyses of the livers of two seals examined; levels were 30-50 times higher than those found in the liver of a monk seal that had been maintained in captivity for 15 years. However, radioimmunoassays for ciguatoxin were not consistent with the bioassay results: one seal had liver ciguatoxin levels 25% above those in the captive seal, and the second had levels 9% below the captive seal. Two of 18 live seals sampled had elevated white blood cell counts. One of these seals disappeared within 2 weeks of sampling, but the other remained healthy at least through 1979.

1.4.2 Hawaiian Monk Seal UME, Laysan Island 2000-2001.

An Unusual Mortality Event was declared in the spring of 2001 following the death of four juvenile monk seals in a 9-day period at Laysan Island. Criteria used by the Working Group on Unusual Marine Mammal Mortality Events (WGUMMME) included increased mortality compared to prior records, mortalities occurring in a localized area, mortalities associated with abnormal behavior patterns, and the mortality of a critically endangered species (Wilkinson, 1996; Antonelis et al., 2001). An investigation was launched at Laysan Island and other subpopulations in the NWHI to assess possible rangewide effects. Survival from weaning to 1 year in 2001 was lower than most previous years at all but one site. Investigation into causes included infectious diseases, parasitism, environmental conditions, naturally occurring biotoxins, and anthropogenic contaminants. Necropsy results from 11 seals revealed emaciation but no signs of infectious disease or toxicosis linking the UME deaths. The unusually high mortality in yearling seals was not correlated with their size at weaning, but appeared to be a result of their inability to forage successfully during the post-weaning transition to nutritional independence (Antonelis et al., 2001). High juvenile mortality was also observed in 2002 (NMFS unpublished data).

1.5 Background of this Document

The first attempt to develop a response plan for UMEs in Hawaiian monk seals is presented by Gilmartin (1987). The National Marine Fisheries Service (NMFS) has published a National Contingency Plan for responding to unusual marine mammal mortality events (Wilkinson, 1996). The WGUMMME recommended that a separate plan, consistent with the national plan and other relevant documents such as the Hawaii Area Oil Spill Contingency Plan, be prepared for Hawaiian monk seals because of their endangered status and the logistic difficulties associated with the remoteness of most of their colonies. A separate plan was prepared for manatees by Geraci and Lounsbury (1997), and the Hawaiian monk seal contingency plan follows the manatee format.

2. Federal/State Authority and Jurisdiction

The Marine Mammal Health and Stranding Response Act was passed by the U.S. Congress in 1992 (P.L. 102-587, 16 U.S.C. 1421 [c-d]), and it became Title IV of the Marine Mammal Protection Act (MMPA). Section 404 of MMPA Title 4 (P.L. 103-238, §24(b)(1)) established a framework for responding to Marine Mammal UMEs, described in detail in the National Contingency Plan for Response to Unusual Marine Mammal Mortality Events (Wilkinson, 1996). The response sequence is outlined below and is presented as a flowchart in Appendix I-1.

- An increase in stranding rates or unusual findings is detected through one of three likely mechanisms:
    - NMFS Regional Stranding Coordinator (Pacific Islands Regional Office, PIRO) detects increased strandings or unusual findings upon reviewing Level A data and consulting with the Stranding Network;
    - Stranding Network directly notifies the NMFS Regional Stranding Coordinator (PIRO) of an increase in strandings or unusual findings;
    - Monk Seal Research Leader observes an increase in strandings or unusual finding and notifies the Leader of the Pacific Island Fisheries Science Center's Marine Mammal Research Program (MMRP).
- The NMFS Regional Stranding Coordinator (PIRO) or the Leader of MMRP will contact the Executive Secretary for the WGUMMME (NMFS Headquarters), pass on the information, and request a consultation with the WGUMMME.
- The Executive Secretary for the WGUMMME will formally request a consultation and forward a complete summary of the event in question and a historical record of stranding data to the WGUMMME.
- Over a 24-hour period, the WGUMMME will review the data and compare it against the seven criteria for determination of an UME (Wilkinson, 1996:18):

- A marked increase in the magnitude of strandings is occurring when compared with prior records. There is no set formula for determining what magnitude would trigger a response. The NMFS Southeast Region has used a formula of the historic mean plus two times the standard deviation to determine a threshold level. The WGUMMME stated that the magnitude must be weighed against other knowledge. As a pragmatic method, it was suggested that if a pulse in strandings is spread over an area or timeframe that strains the capacity of the Stranding Networks to respond, it should be cause for concern.

- Animals are stranding at a time of the year when strandings are unusual.

- An increase in strandings is occurring in a very localized area (possibly suggesting a localized problem), is occurring throughout the geographical range of the species/population, or spreads geographically with time.

- The species, age, or sex composition of the stranded animals is different than those of animals that normally strand in the area at that time of year.

- Stranded animals exhibit similar or unusual pathologic findings, or the general physical condition (e.g., blubber thickness) of stranded animals is different from what is normally seen.

- Mortality is accompanied by behavior patterns observed among living individuals in the wild that are unusual, such as occurrence in habitats normally avoided or abnormal patterns of swimming and diving.

- Critically endangered species are stranding. Stranding of three or four right whales, for example, may be cause for great concern whereas stranding of a similar number of fin whales may not.

- The WGUMMME will return its decision to the Executive Secretary, and an official determination will be made if a quorum has been reached (i.e., majority of 2/3 of the voting body).

- The Executive Secretary officially requests concurrence from the NMFS Assistant Administrator. With this concurrence, the NMFS Assistant Administrator is also requested to appoint an On-Site Coordinator (see below).

3. The On-Site Coordinator

3.1 The Appointment of the On-Site Coordinator.

Following the sequence of events described above, the NMFS Assistant Administrator will appoint an On-Site Coordinator for the UME. The On-Site Coordinator is the appropriate NMFS Regional Administrator or his or her designee (Wilkinson, 1996). The

selection of On-Site Coordinator is based upon recommendations from the WGUMMME and the Leader of the MMRP, PIFSC, NOAA Fisheries.

3.2. Responsibilities of the Federal On-Site Coordinator.

The On-Site Coordinator should have strong management and leadership abilities, strong communication skills, the ability to make decisions with minimal use of intermediaries, the ability to access information and expertise (including interagency expertise), familiarity with the National Contingency Plan and associated protocols (including this monk seal contingency plan), and familiarity with and the ability to work with the Marine Mammal Stranding Networks (Wilkinson, 1996:19). The On-Site Coordinator must hold or be included on a federal Enhancement and Scientific Research Permit (e.g., the permit held by the Leader of MMRP).

The On-Site Coordinator will work closely with the Executive Secretary, NMFS national and regional offices (e.g., PIRO) and the Leader of MMRP. The On-Site Coordinator also will notify other federal agencies (e.g., U.S. Coast Guard, U.S. Fish and Wildlife Service), State of Hawaii wildlife resource agencies (e.g., Hawaii Department of Land and Natural Resources, Division of Aquatic Resources, and Division of Forestry and Wildlife,), Hawaii regional stranding network members, and the Hawaiian Monk Seal Recovery Team. A list of federal and state agencies and individuals to be notified is included in Appendix H-1. Contact lists are updated regularly (at least annually) by MMRP. Additional notifications will be made as needed; for example, the Hawaii Department of Public Health would be notified if a serious human health hazard (e.g., if a serious zoonotic disease is identified or suspected) existed on one of the main Hawaiian Islands, and USDA/APHIS would be notified if monk seal transport were being considered. The On-Site Coordinator will also assemble a response team as described below.

4. Pre-Event Planning for Rapid Response

4.1 Building a Response Team

The response team assembled by the On-Site Coordinator will include one or more wildlife biologists with knowledge of normal Hawaiian monk seal behavior and natural history and one or more veterinarians familiar with pinniped diseases and monk seal sampling protocols (including specimen collection and necropsy protocols). Additional team members will be selected depending on the size and nature of the event and may include a veterinary technician to assist with sample collection and processing, a rescue and salvage logistics coordinator, a parasitologist, and an environmental sampling coordinator. A list of potential team members is included in H-2; this list is updated at least annually by MMRP to ensure that a qualified team can be assembled immediately.

4.2 The Administrative Support Team

Three important administrative areas to be addressed by the On-Site Coordinator in the event of a Hawaiian monk seal UME are financial administration, public information

coordination, and tracking of animals and samples. As stipulated in the National Contingency Plan (Wilkinson, 1996), a certified Contracting Officer's Technical Representative will be appointed as financial officer by the Regional Office and will work with the On-Site Coordinator and a certified Contracting Officer to negotiate and enter into agreements for services performed in the course of a MMUME response, to locate and order supplies and equipment, and to be responsible for prompt payment for services, purchase orders, and expense reimbursements. The roles of On-Site Coordinator and Contracting Officer's Technical Representative can be filled by the same individual or by two separate individuals. The On-Site Coordinator will appoint a local media contact (either an agency public affairs officer or an individual involved in the response) to address public concerns and inquiries and to keep the NMFS Office of Public Affairs (301-713-2370) informed. Procedures for tracking data and samples will follow well-established protocols (Monk Seal Specimen Collection and Necropsy protocols, Appendices A and B). In some cases (e.g., potential litigation or prosecution related to the MMUME) chain of custody will be maintained for all samples and data collected during the response (Appendix G-6).

4.3 Scientific Advisors

In addition to the WGUMMME, the On-Site Coordinator may call on scientific advisors from the Hawaiian Monk Seal Recovery Team, the Marine Mammal Commission, or the list of attendees to the 11-12 September 2000 Hawaiian Monk Seal Health Studies Workshop. Additional advisors may be consulted depending on the size and nature of the event (e.g., the NOAA Marine Biotoxins Program, 843-762-8500, will be contacted if a Harmful Algal Bloom is suspected).

4.4 Training and Readiness

MMRP conducts extensive annual training of field personnel for the monk seal assessment program. Many of the topics addressed in this training program are relevant to MMUME response, such as the Monk Seal Health and Disease Program (including procedures and supply lists for specimen collection, necropsies, and field laboratory assays (Appendices A through H), field camp logistics and set-up, animal care and use policies, field communications and safety, and first aid training. In addition to this pre-deployment training, field camp personnel have practical experience in Hawaiian monk seal data collection and processing obtained during deployments of 2-5 months in the NWHI.

Monk seal rescue and rehabilitation are addressed in the Monk Seal Rehabilitation Manual (Appendix D). Lavigne (1999) reviewed past attempts to enhance monk seal survival and reproduction via the "headstart program," the rescue, rehabilitation and transport of undersized females and the translocation or removal of adult males to reduce mobbing. Such efforts have had varying levels of success in enhancing monk seal survival.

The Leader of MMRP (Dr. George Antonelis, 808-983-5710) has designated individuals for key roles such as monk seal population assessment (Jason Baker, 808-983-5711) and health studies (Dr. Robert Braun, 808-254-8181 or 808-254-3530 or 808-783-6565; Lizabeth Kashinsky, 808-592-8306) with well-defined lines of authority and responsibilities. Members of the Hawaii Marine Mammal Stranding Network have responded to events such as monk seal entanglements in fishing gear, vessel grounding, and the presence of females and pups on public beaches in the main Hawaiian Islands. The Hawaiian Islands Response Group V.P. (Marlee Breese, 808-259-5268 or 808-291-6434) is a key contact in the stranding network of Hawaii.

4.5 Establishing Memoranda of Understanding (MOUs) with Cooperators

The U.S. Navy and the U.S. Coast Guard have provided logistical support to the Marine Mammal Research Program in past years on a not-to-interfere basis. This support has included transportation (of personnel, equipment, and supplies) to and from the NWHI and medical evacuation of injured field personnel. MOU with these agencies are being explored to establish formal mechanisms for support in the event of a monk seal UME. Additionally, an Oil Spill Response Plan is in the final stages of review and will be available soon.

4.6 Pre-Planning for Sample Analyses

The Marine Mammal Research Program has designated several laboratories (Appendix H-3) for analyses of samples collected from Hawaiian monk seals. In addition, an epidemiology sampling program provides useful baseline data for comparison in the event of a MMUME (Aguirre et al., 1999; Aguirre, 2000). The epidemiology sampling program is reviewed periodically (e.g., September 11-12, 2000 Hawaiian Monk Seal Health Studies Workshop; November 2001 conference call coordinated by Dr. Robert Braun, monk seal health studies coordinator) and changes are implemented as needed (cf. Appendix E-1; following the 2000-2001 abortion investigation at Laysan Island, sampling and necropsy protocols were modified to improve the efficiency of UME investigations).

5. Recognizing an Unusual Event

The following criteria for defining a MMUME are given in the Marine Mammal Health and Stranding Response Act (Wilkinson, 1996; Dierauf and Gulland, 2001):

- It is unexpected;
- It involves a significant die-off of a marine mammal population; or
- A small number of a severely endangered marine mammal species appear to be affected;
- Demands an immediate response.

During the 2000 abortion investigation at Laysan Island, the following additional criteria were used: increased mortality compared to prior records, mortalities occurring

in a localized area, and mortalities associated with abnormal behavior patterns (Antonelis et al., 2001).

5.1 Guidelines for a Graduated Response

Current Hawaiian monk seal research activities include population monitoring, epidemiology program sampling (live and dead seals), documentation of injuries (e.g., wounds caused by conspecifics or predators) that might affect an individual seal's survival, monk seal feeding habits studies, and documentation of the use of marine habitats by monk seals using satellite-linked telemetry and underwater cameras ("CRITTERCAM"). Research conducted as part of the Hawaiian Monk Seal Assessment has generated baseline data on expected numbers and timing of births and deaths at monk seal colonies; long-term tagging and monitoring programs have generated individual life history data for approximately 90% of the population. The Hawaiian Monk Seal Health and Disease Program maintains a frozen serum bank and a biomedical database indexed by island and by individual seal. NMFS field camp personnel and others (e.g., USFWS refuge biologists) are trained to recognize and report live monk seals exhibiting abnormal behavior or appearance, as well as any dead seals encountered to the Marine Mammal Research Program Leader (Dr. George Antonelis) and the program's designated veterinarian and coordinator of the monk seal health studies (Dr. Robert Braun). Early recognition and rapid reporting of a potential MMUME provide time to alert key individuals and cooperators (including the NMFS Regional Office, NMFS National MMUME Coordinator, and the NMFS Office of Protected Resources), conduct preliminary studies and gather additional data, assess capability for response, open lines of communication, and identify and refine (as necessary) protocols for response (Geraci and Lounsbury, 1997).

Baseline data collection and population monitoring of other species occurs seasonally (in some cases, year round) in the NWHI and may assist in early recognition and reporting of an UME. Fish censuses are conducted annually (and in some cases, monthly) in many locations in monk seal habitat as part of ongoing coral reef research programs. The U.S. Fish and Wildlife Service conducts surveys at least annually of sea turtle nesting activity and abundance and distribution of resident and migratory birds. Year-round camps are maintained at some locations in the NWHI (e.g., Midway Atoll, Laysan Island, FFS), staffed by federal and state wildlife biologists. An ongoing photo-identification project is documenting occurrence and behavior of *Stenella longirostris* at Midway Atoll, Kure Atoll, and Pearl and Hermes. The NMFS Southwest Fisheries Science Center is surveying cetacean populations along transects near the Hawaiian Archipelago. An increase in the number of stranded marine mammals or sea turtles would be detected by the NMFS Regional Stranding Coordinator.

5.2 Protocol for Initiating Unusual Event Designation

As specified in Title IV of the Marine Mammal Health and Stranding Act, the NMFS Pacific Area Protected Species Program Coordinator/Regional Stranding Coordinator (or his or her designee; e.g., the Leader of MMRP) will contact the NMFS National

MMUME Coordinator if dead or moribund monk seals are observed unexpectedly. This contact initiates a chain of events illustrated in a flow chart by Dierauf and Gulland (2001, Fig. 1, p. 75), reprinted as Appendix I-1 and briefly summarized here. The NMFS National MMUME Coordinator will alert the members of the WGUMMME, who will require information on the background levels of morbidity and mortality to decide whether or not a MMUME is taking place (data to be obtained from prior records; Wilkinson, 1996; p. 18). For example, is the age and sex composition, behavior or body condition of the stranded seals different from what occurs normally in that geographic location or at that time of year? If the Working Group decides that a MMUME is occurring, the MMUME National Coordinator informs the Regional Stranding Coordinator, designates an On-Site Coordinator (though the Secretary of Commerce), and transfers responsibility for action to the On-Site Coordinator.

6. Notification of Personnel and Agencies

The On-Site Coordinator will notify and mobilize federal, state, and other authorized Hawaiian monk seal rescue and recovery program personnel, possible Response Team members, and pre-identified laboratories and MOU cooperators promptly. The On-Site Coordinator also will notify other federal agencies, state wildlife resource agencies, local government agencies, stranding network personnel and the Leader of the Monk Seal Recovery Team (see Section 3.2 above). Contact information for agencies and individuals to be notified are included in Appendices H-1 through H-4; these lists are updated at least annually by MMRP. Additional notifications of other stakeholders will be made as needed; for example, the Hawaii Department of Public Health will be notified if a serious human health hazard exists on one of the MHI (e.g., if a serious zoonotic disease is identified or suspected).

7. A Tailored Response

7.1 Objectives and General Guidelines

The On-Site Coordinator will work with the WGUMMME to determine and initiate a course of action to protect the public health and welfare, identify the cause(s) of the event (including possible contributing factors such as malnutrition or contaminants), minimize deaths and provide for the rehabilitation of individual animals, and determine the impact of the MMUME on the population (Wilkinson, 1996; Geraci and Lounsbury, 1997; Dierauf and Gulland, 2001).

7.2 Responding to an Event of Known Cause

Wilkinson (1996, Table 1; Appendix I-3) and Geraci and Lounsbury (1997) describe the steps to be taken (including continuing consultation with the WGUMMME) in response to a MMUME of known cause. Chain of custody procedures will be implemented for all data and samples collected in response to a MMUME (Wilkinson 1996; Appendix G-6), even if the cause is already known or suspected. Pre-identified risks to Hawaiian monk seals include biotoxins such as ciguatera, anthropogenic

chemical spills, abortion "storms," viral epizootics (e.g., morbillivirus), and human-related trauma (e.g., entanglement).

- **Naturally occurring toxins (e.g., harmful algal bloom, ciguatera)**. The NOAA Marine Biotoxins Program has posted a Flow Diagram for Suspected Marine Biotoxin Incidents (www.chbr.noaa.gov/CoastalResearch/Pictures/AnalyticalResponseTeam/FlowDiagram.gif); this will be used to guide response to a suspected harmful algal bloom. Specimen collection protocols for specific biotoxins are presented in Rowles et al. (2001:468, Table 5). See Appendix E-2.
- **Anthropogenic chemicals (e.g., oil or other chemical spill)**. Procedures will be consistent with the contingency plans developed by the U. S. Coast Guard (28 August 2001 update of the Hawaii Area Contingency Plan, www.uscg.mil/d14/units/msohono/HACP1/index.htm; Oil Spill Field Operations Guide, www.uscg.mil/d14/units/msohono/ics/fog/index.htm). Specimen collection protocols for chemical pollutants are presented in Rowles et al. (2001:466; Table 4). See Appendix E-3.
- **Abortion**. Four aborted fetuses were observed over a 4-week period at Laysan Island in 2000. It is not known if these carcasses represented a true increase in mortality or simply an increase in survey effort. Baseline data on morbidity and mortality are sparse for this time of year because NMFS field camps at Laysan Island typically are established several weeks later. Results of the investigation into this event were inconclusive. Sixteen seals were sampled, including one adult female thought to have aborted a fetus. Laboratory results (including tests for *Chlamydia* sp., *Brucella* sp., *Leptospira* sp., morbillivirus and herpesvirus) were consistent with baseline serologic test results obtained during previous epidemiology studies on Hawaiian monk seals. Five placentas (from surviving pups) were examined and considered to be normal. No gross lesions were noted during one post-mortem exam on an adult female; histopathology revealed only changes consistent with advanced age. However, because abortion-causing organisms are a recognized threat to monk seals, genital swabs were added to the standard epidemiology and necropsy sampling protocols in 2001 (cf. Appendix E-1).
- **Infectious agents (e.g., morbillivirus)**. Live capture and isolation of affected individuals (e.g., while laboratory analyses are pending) may be necessary if an epizootic is suspected. Osterhaus et al. (1998) proposed that vaccination be considered in the management and conservation of Mediterranean monk seals. The Marine Mammal Research Program is reviewing the potential risks and benefits of morbillivirus vaccination in the face of an outbreak in Hawaiian monk seals.
- **Human-related trauma (e.g., entanglement)**. Chain of custody procedures (Appendix G-6) will be implemented for all data and samples collected in response to an MMUME involving human-related trauma. In addition, NMFS Evaluation of Human Interaction forms (Appendix G-5) will be completed for affected animals.

7.3 Investigating an Event of Unknown Cause

Wilkinson (1996, Table 1; Appendix I-3) and Geraci and Lounsbury (1997) describe the steps to be taken (including continuing consultation with the WGUMMME) in response to a MMUME of unknown cause. Initial data collection will follow existing monk seal protocols (e.g., Appendices A through D, G and H) to ensure that information collected during the MMUME are comparable to baseline data. As the event progresses and at the recommendation of the WGUMMME, additional sampling protocols may be implemented (cf. Appendix E).

If no cause is readily apparent, the investigation may include analyses of other species in the area of the monk seal UME in order to identify or validate possible causes (see Section 5.1 for a description of monitoring programs for other species). For example, harmful algal blooms often affect a number of plantivorous and piscivorous species (e.g., crustaceans, fish, seabirds, sea turtles). The Marine Mammal Research Program is preparing cooperative agreements with agencies that oversee these species and resources (e.g., USFWS Refuge Managers, NOS-NWHI Coral Reef Reserve, State of Hawaii Department of Land and Natural Resources, NOAA National Marine Sanctuary Program personnel) to expedite such investigations in the event of a monk seal UME.

Under certain conditions (Wilkinson, 1996; p. 41), and if it does not inhibit the ongoing investigation, NMFS may accommodate requests from independent researchers for tissue samples and data to address other scientific questions that may or may not be related to the MMUME. Chain of custody procedures will be implemented for all data and samples collected in response to a MMUME (Wilkinson, 1996; Rowles et al., 2001:452 [Fig. 1]).

7.4 Determining "The End"

The WGUMMME, in consultation with the On-Site Coordinator, will determine when a MMUME has ended. The end point of a MMUME may be as difficult to pinpoint as the onset, particularly if the decline in morbidity or mortality is gradual. If there is a seasonal component to the event (e.g., an abortion 'storm'), investigators may not be convinced that the MMUME is over until the following year, when it is possible to assess whether or not values (e.g., number of premature pups observed) have returned to historic or baseline levels (when compared to prior records, cf. Wilkinson, 1996; p. 18).

7.5 Post-Event Assessment and Monitoring

Once the end point of a MMUME has been determined, the On-Site Coordinator will prepare a report for submission to the WGUMMME containing the results of all analyses conducted during the investigation, an assessment of the impact of the MMUME on the Hawaiian monk seal population, and recommendations for future monitoring (Wilkinson, 1996). In some cases, post-event monitoring will return to pre-event levels (i.e., the Monk Seal Assessment and Health and Disease programs of MMRP). In other cases, protocols

may be modified (cf. Appendix E) and additional recovery or population monitoring activities may be incorporated into existing research and management programs.

8. Communication and Reporting

The On-Site Coordinator must maintain a regular communication and reporting schedule with the Response Team Leader and the WGUMMME. Telephone or email briefings every 1 to 2 days will enable the On-Site Coordinator to recognize and address personnel, equipment, supply, and data needs of the response team and to ensure that critical data and samples are being collected. The Response Team Leader should submit a brief written (e-mail) report to the On-Site Coordinator once a week, summarizing the status of the investigations and noting unresolved problems. The On-Site Coordinator should forward this report with whatever additional calculations, maps, histograms, or laboratory analysis results are available to the WGUMMME. The On-Site Coordinator and WGUMMME may invite additional experts to review and discuss these reports and will determine what additional efforts are needed to respond to and investigate the MMUME.

The On-Site Coordinator will also provide regular official reports on the progress of the investigation to appropriate federal, state, and local agencies and to the Hawaiian Monk Seal Recovery Team. The media contact appointed by the On-Site Coordinator will keep the NMFS Office of Public Affairs informed, prepare press releases as needed, respond to media requests for interviews, and address public concerns and inquiries.

9. Public Health Concerns

Public health and welfare are the first priorities in responding to a MMUME (Wilkinson, 1996). The On-Site Coordinator will ensure compliance with safety guidelines (e.g., § 300.150 of the National Oil and Hazardous Substances Pollution Contingency Plan [40 CFR Part 300] specifies the type of training required for personnel responding to an event involving oil discharges or hazardous chemical releases).

Only properly trained and equipped personnel will participate in a MMUME response. Safety training programs conducted annually for field personnel working with Hawaiian monk seals include animal handling skills, first aid, and hygienic precautions such as the Monk Seal Clean Protocol and hazardous waste procedures. Additional training (e.g., boat handling, aviation safety) will be provided as needed.

Carcasses may be left on the beach to decompose naturally unless a transmissible pathogen or serious toxin is suspected. In this case, the carcass may be buried, taken to a sanitary landfill, or incinerated.

If it is necessary to isolate or quarantine live animals during a MMUME, existing protocols developed for Hawaiian monk seals will be implemented (Appendix D).

10. Resources: Location and Utilization

10.1 Introduction

Although Hawaiian monk seals do occur on the MHI, the majority are found on or around the NWHI, a chain of islands and atolls extending hundreds of kilometers. Some of these sites are accessible only by boat, and weather and sea conditions often prevent landing. Time to mount a response to a monk seal UME is likely to range from a few hours (e.g., Kauai) to a week or more (e.g., Laysan Island).

The presence of other protected species (e.g., nesting seabirds) may affect the activities of UME response personnel. For example, U.S. Fish and Wildlife Service quarantine protocols are in effect at some islands requiring special care and treatment of clothing, footwear, equipment, and supplies. Access to island interiors may be restricted at some islands to prevent disturbance of sensitive animal or plant species.

10.2 Equipment and Logistics

All supplies and equipment needed for a limited MMUME response are maintained at the Kewalo Research Facility in Honolulu, including capture and handling gear (including supplies and equipment for chemical immobilization and emergency cardiopulmonary resuscitation), biomedical sampling kits for up to 50 monk seals, and a portable laboratory. Equipment lists are included in Appendix F.

Charter flights can be arranged to Midway Atoll, Tern Island, or FFS. Vessel charters can also be arranged for logistical support. A list of charter companies is maintained by MMRP. The U.S. Coast Guard (Office of Aids to Navigation for ship transport, CGAS Barbers Point for air transport) and U.S. Navy have provided emergency transport and equipment drops on occasion. MOU with these agencies are being explored to establish formal mechanisms for support in the event of a monk seal UME. The NOAA ship *Oscar Elton Sette* and other NOAA research vessels could be diverted from other activities near the Hawaiian Archipelago to support a monk seal UME investigation if approved by the Director of PIFSC or the Sanctuary Office.

10.3 Information and Data Resources

Contact information for key personnel (e.g., potential Response Team members) is included in Appendix H. MMRP maintains current contact lists and protocols, and the monk seal assessment program maintains a database of relevant historical information such as population counts (beach counts, aerial surveys) and pup mortality rates. Up to about 90% of all seals in the NWHI are individually identified each field season. Reproductive histories are known for a majority of females at Laysan and for many seals at Lisianski and FFS. Age-specific survival is well known for nearly all animals born in the past 20 years or so. The Monk Seal Health and Disease Program maintains a baseline biomedical database for Hawaiian monk seals, including hematology and serum biochemistry reference ranges, seroprevalence of potential pathogens, gross necropsy and

histopathology reports, and parasite surveys (cf. Aguirre, 2000). Individual biochemical serum test results are available for almost 25% of the population.

10.4 Development of Temporary Animal Housing and Emergency Facilities

Rehabilitation facilities for Hawaiian monk seals are maintained at the NMFS Kewalo Basin site (pools sufficient to hold 3-5 monk seals), although the ability to isolate seals is limited. The Marine Mammal Research Program has construction plans, equipment and supply lists, and approximate cost estimates for temporary holding facilities that could be erected in remote locations in the NWHI. Gilmartin et al. (in review) describe holding pens (35-60 square meters), incorporating sandy haul-out areas and shallow seawater access.

10.5 Funding

The MMUME Fund, established in Title IV of the Marine Mammal Protection Act, is an interest-bearing account in the Federal Treasury to be used exclusively for costs associated with preparing for and responding to MMUMEs (Dierauf and Gulland, 2001). In the event of a MMUME, funds are made available through the National Contingency Plan (Wilkinson, 1996; Dierauf and Gulland, 2001). Post-UME monitoring is not supported by the MMUME Fund, but other federal sources (e.g., Prescott Grant) are available for possible funding.

11. Response and Investigation Protocols

11.1: Rescue and rehabilitation (Appendix D)

11.2: Release of rehabilitated monk seals after a MMUME

Section 402(b) of the Marine Mammal Protection Act contains guidelines for release of rehabilitated animals. The WGUMMME will be consulted to determine whether additional event-specific release criteria are needed. The health of the wild population will take precedence over the health or fate of an individual monk seal (Wilkinson, 1996).

11.3: Procedures for Carcasses (Appendix B)

11.4: Tissue Sample Collection (Appendices A, B, G)

11.5: Environmental Assessment and Sampling

Environmental data for the Hawaiian Islands (e.g., sea surface temperature, wind speed, wave height, and primary productivity) are collected by a variety of national and international scientific agencies. Wind data used for estimates of oceanic (Ekman) transport and ocean mixing (Reynolds) for the period of August 1991 through January 2001 come from the Advanced Microwave Instruments on ERS-1 and ERS-2 spacecraft. These data are provided courtesy of the European Space Agency. The source of wind

data since January 2000 is the Seawinds sensor on the QuikSCAT spacecraft, provided courtesy of the NASA Jet Propulsion Laboratory (an element of the California Institute of Technology). The multichannel sea surface temperature (MCSST) data set was developed by the University of Miami and is distributed by the NASA Jet Propulsion Laboratory PODAAC (JPL PODAAC) administered by the California Institute of Technology. Altimetry data also are available from JPL PODAAC. Near real-time data were provided by the NOAA/NESDIS Office of Research Applications until 2001; these data are now available from the U.S. Navy Fleet Numerical Meteorological and Oceanographic Center in Monterey, California. Chlorophyll *a* data will be obtained through the SeaWIFS and MODIS, NASA Active Archive Center at the Goddard Space Flight Center.

11.6: Packaging and Shipping Samples (Appendices A and H)

11.7: Keeping Records and Maintaining Chain of Custody (Appendix G-6)

11.8: Surveys

Hawaiian monk seal population assessment field research is conducted at the six main breeding subpopulations each year. During this field effort, researchers conduct regular surveys of the monk seal's terrestrial habitat, noting information on individual animal identity, location, size class, sex, coarse body condition, and any other notable information such as reproductive condition, nursing pair associations, molt status, wounding, apparent illness, mortalities, etc. Field research is typically conducted on the following schedule:

Laysan I., Lisianski I., Midway Atoll: mid-March to mid-July

Kure Atoll, Pearl and Hermes Reef: mid-May to mid-July

French Frigate Shoals: mid-May to mid-August

At Laysan and Lisianski Islands, researchers conduct daily surveys of the island perimeter. At the remaining multiple-islet atolls, surveys are typically conducted at least weekly, weather permitting.

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14. List of Appendices

A. HAWAIIAN MONK SEAL SPECIMEN COLLECTION

B. HAWAIIAN MONK SEAL NECROPSY PROTOCOL

C. EQUIPMENT AND SUPPLIES FOR A SINGLE NECROPSY

D. HAWAIIAN MONK SEAL RESCUE AND REHABILITATION GUIDELINES

E. ADDITIONAL SAMPLE COLLECTION PROTOCOLS

1. Additional Samples Requested during the Laysan 2001 MMUME
2. Specimen Collection Protocols for Specific Biotoxins (Rowles et al., 2001: 468)
3. Specimen Collection Protocols for Chemical Pollutants (Rowles et al., 2001: 466)

F. EQUIPMENT/SUPPLIES CHECKLISTS

1. Epidemiology Sampling Supply List
2. Veterinary Medical Kit

G. SAMPLE FORMS

1. Monk Seal Specimen Collection Summary Form
2. Hawaiian Monk Seal Tagging & Chemical Immobilization Form
3. Hawaiian Monk Seal Clinical Examination Form
4. Hawaiian Monk Seal Field Hematology Report
5. NMFS Human Interaction Forms
6. Marine Forensics Chain of Custody Form (Rowles et al., 2001. Gross Necropsy and Specimen Collection Protocols. Pp. 449-470 in Dierauf, L.A. and F.M.D. Gulland [eds] CRC Handbook of Marine Mammal Medicine, CRC Press, Boca Raton; Figure 1, p.452).

H. IMPORTANT CONTACTS

1. Federal, State and Local authorities
2. Potential Response Team members
3. Diagnostic Laboratories
4. Cooperating Experts

I. FLOW CHARTS

1. Flowchart and timing of response to MMUME in the United States
2. Flow Diagram for Suspected Marine Biotoxin Incidents (NOAA Marine Biotoxins Program)
3. Flow chart for response to a marine mammal mortality event with known vs. unknown cause (Table 1 in Wilkinson, D.M. 1996. National Contingency Plan for Response to Unusual Marine Mammal Mortality Events. NOAA Tech. Memo. NMFS-OPR-9, 118 p.)

J. SAMPLE MOUs FROM CONTINGENCY PLAN FOR MANATEE DIE-OFFS

1. MOU with participants in UME response
2. MOU with laboratories/individuals performing tests/analyses

K. HAWAII AREA OIL SPILL CONTINGENCY PLAN

1. Excerpts from 28 August 2001 Update of the Hawaii Area Contingency Plan (www.uscg.mil/d14/units/msohono/HACP1/index.htm)
2. Excerpts from Oil Spill Field Operations Guide (www.uscg.mil/d14/units/msohono/ics/fog/index.htm)

APPENDICES

APPENDIX A:

Hawaiian Monk Seal Specimen Collection Protocol

Jan 29 2003/RCB

HAWAIIAN MONK SEAL SPECIMEN COLLECTION PROTOCOL

Introduction

The following protocol describes the collection, processing, preservation, packaging, and shipment techniques for specimens collected from Hawaiian monk seals. Strict adherence to this protocol is necessary to ensure that each specimen is handled and preserved in an appropriate and standardized manner. There is an optimal chance to determine the cause and nature of disease and to assess its significance, and the resulting analyses are valid and useful to the management of this endangered species. Additional references are provided in Section IV.

SPECIMEN COLLECTION: POPULATION ASSESSMENT FIELD CAMPS

Specimens to be collected during population assessment work include:

Scats
Spews
Endoparasites
Skin tissue plugs
Molt samples
Placentas
Ticks
Skulls

Additional specimens to be collected during assessment work include specimens collected from necropsies (see **Hawaiian Monk Seal Necropsy Protocol**). Strict adherence to the necropsy protocol is necessary in order to obtain the appropriate specimens from dead monk seals.

I. Specimen Labeling

All specimen labels must include the following information:

Seal ID
Date collected (YYMMDD format)
Specimen Type/ Sub-type
Specimen #/sub #, Island/Atoll
Include the following if there is room (container larger than cryovial):
Seal size and sex (if known)
Preservation method
Collector initials

When filling out labels, **please write legibly** and distinguish between "zero" and the letter "0" by drawing a line through the zero. Unless noted otherwise, **all** specimens should have both an inner and outer label.

Inner labels

1. Specimens in jars, glass vials, whirlpaks, and ziplocs should have preprinted waterproof labels inside containers in case the outer labels fall off or become illegible. Fill out the labels with pencil and **be sure to position them inside containers so that labels can be read from the outside** without having to remove the labels to read them.

2. Specimens in cryovials (blood, serum, plasma, certain blubber samples, etc) and teflon vials **should not** have inner labels.

Outer labels

1. Jars and glass vials: use preprinted stick-on specimen labels. After filling out the label, seal with clear tape.

2. Whirlpaks and ziplocks: write directly on bags with a sharpie.

3. Cryovials: write directly on vial using cryoware markers (provided in necropsy kits). Use the following markers for all **epidemiology samples**:

black marker=serum
red marker=plasma LTT
green marker=plasma GNTT
blue marker=buffy coats

4. Teflon vials: use pencil to write on the special labels provided with the vials, then put in the vials in whirlpaks with labels both in and out of whirlpaks.

II. Specimen Preservation

Refer to material safety data sheets (MSDS) for health effects, first aid, and toxicity of chemicals.

Common Preservation Methods

PRESERVATION	CODE	SPECIMEN	FORMULA
Dry	DR	scats, molts	n/a
95-100% ethyl alcohol	AA	tissue plugs, molts	n/a
AFA	AF	parasitology	50 ml 95% ethyl alcohol (not denatured), 5 ml of acetic acid, and 45 ml of 10% formalin (or 10 ml formaldehyde and 35 ml of water
Alcohol/glycerol solution (70 + 5)	AG	parasitology	95 ml of 70% ethyl alcohol (not denatured) and 5 ml glycerin
70% ethyl alcohol	AL	parasitology	265 ml fresh water to 735 ml of 95% ethyl alcohol
Polyvinyl alcohol	PV	parasitology	supplied in pre-filled vials
10% buffered formalin	FM	placentas, necropsy tissues	100 ml 37% formaldehyde, 800 ml fresh water, and 100 ml seawater
Liquid nitrogen	LN	necropsy tissues, spew, molts, ticks,	

Note: when placing items in liquid nitrogen dewar, **BE SURE** items are small enough to be removed from dewar!

NEVER STORE SPECIMENS IN REFRIGERATOR/FREEZER USED FOR STORING FOOD FOR HUMAN CONSUMPTION

III.SPECIMEN COLLECTION METHODS

A. SCATS

Scats are collected for prey species determination and parasite studies. Collect a minimum of 100 scats at each island or atoll. **Always wear disposable latex gloves and avoid direct contact with feces**. Select fresh scats (not hard or chalky white) and eliminate as much extraneous material as possible (e.g., sand, coral, leaf litter, etc.) Emphasis should be on quality, not quantity. In addition, collect a minimum of 10 and a maximum of 30 PVA fixed scat sub-samples from **fresh scats from known animals. Collection efforts should be focused on samples from juvenile animals and collection of repeat samples from the same individuals**. For collection methods, see parasitology section below.

1. If endoparasites are observed, collect immediately. Rinse, relax, fix, and store as described in the parasitology section below. Be sure parasites are assigned the same specimen number as the scat from which it is derived, but give each specimen a unique specimen sub-number.

2. Collect entire scat (or what's remaining if sub-sample(s) for parasitology are taken) in whirlpaks or ziplocs. Be sure to include legible specimen labels both inside (using waterproof labels) and outside the bags. Take care to ensure that the inside labels are visible from the outside.

3. Once any parasitology sub-samples have been collected, add 20% liquid detergent, 80% sea water solution to scat sample to begin the degreasing process and to kill some of the pathogens. Add just enough of this solution to keep samples moist. Remove as much air as possible from bags before storing them in buckets. Be sure the samples are stored double bagged and are kept upright in buckets to prevent leaks.

B. SPEWS

Spews (vomitus) are collected for prey species determination and parasite studies. They may also be used to detect ciguatera (a naturally occurring biotoxin found in dinoflagellates) in tissues of certain prey species. Spews are often found near seal haul out tracks and may contain partially digested eels, fish, lobster carapaces, octopus, or whole prey items. Note: seabirds also spew squid, flying fish, and other prey species, so be sure to closely observe seal tracks in the area the spew is found and collected.

1. If endoparasites are observed in the spew, collect them immediately and preserve as described in the parasitology section below. Be sure parasites are assigned the same specimen number as the spew from which it was derived, but give each specimen a unique specimen sub-number.

2. Next, collect a **representative sub-sample of spew that is about 300-400 gm (100 gm is approximately** hamburger sized) for ciguatera analysis. Once the representative sample is collected, split this sub-sample up into ~50 gm units and place in whirlpaks. Assign this spew sub-sample the **same specimen number** as the spew from which it was derived, but **give only ONE unique specimen SUB-number to the representative** sample, even though it is being split into multiple bags. In addition to recording specimen number and sub-number on inner and outer labels, note bag number and total number of bags for one sub-sample on each individual bag (1 of 5, 2 of 5, etc.) Be sure to also record the total number of bags for one spew sub-sample in the "notes" field of the specimen collection summary form. **NOTE**: the splitting of the sample is necessary to store the samples in liquid nitrogen. **BE SURE** the split samples will fit easily into and out of the liquid nitrogen dewar. It can be very difficult, if not impossible, to remove large items from the dewars due to their internal design.

3. Once any parasites sub-samples and the ciguatera sub-sample have been collected, store remaining spew **DRY** in a whirlpak or ziplock, double bagged. Clearly label the outside of the bag. Be sure to also include a legible specimen label inside (using waterproof labels) the bag. Arrange the inside label so that it is visible from the outside. No **preservation is necessary.** Be sure that the specimen is packed so that **it is stored upright in buckets for storage and shipment.**

C. PARASITES

Polyvinyl Alcohol (PVA) fixed fecal specimens: Collect a minimum of 10 and a maximum of 30 PVA fixed scat samples from fresh scats from known animals. **Collection efforts should be focused on samples from juvenile animals and collection of repeat samples from the same individuals.**

1. After collecting a fresh scat sample, take a fecal sub sample using the scoop of plastic lid of prefilled vials and/or wooden applicator to collect feces from the center of the scat. Avoid collecting sand and other non-fecal debris in sample.

2. Add sufficient fecal material to PVA vials (prefilled with 5 ml of PVA) to bring the liquid volume of the vial to 7 ml. Using scoop and/or applicator sticks, thoroughly mix fecal material with fixative breaking up as much of the fecal material as possible. Tightly screw cap on, and mix thoroughly by shaking 10+ cycles with rapid twisting motions of the wrist. If you do not have prefilled vials, the proper ratio is one part feces to two parts fixative.

Cestodes (tapeworms) and Trematodes (flukes): Trematodes are flatworms with unsegmented, leaf-shaped bodies. Cestodes are also flatworms, but differ in that they have ribbon-like bodies that are divided into segments called proglottids. These proglottids can be observed with the naked eye in scat samples. To preserve both types, remove all grossly visible parasites using wooden applicator sticks and rinse in a container with sea water or .85% saline solution. Refrigerate or chill on blue ice for at least 4 hours to allow the parasites to relax. After they have become flattened and extended they can be fixed in pre-warmed or hot AFA (alcohol-formalin-acetic acid) for at least 30 minutes (fixatives can be prewarmed by setting out in sun about 1 hour). If parasites are very long, swirl into fixative using applicator stick. After fixing, rinse well with fresh water or 70% ethyl alcohol to eliminate acetic acid that can destroy the specimen. Preserve and store the parasites in AG.

Nematodes (roundworms): Nematodes are round in cross section. To preserve, rinse clean in sea water or .85% saline and fix directly in warm or hot AG.

D. TISSUE PLUGS

After tagging a seal (see "Tagging/Handling Protocol") , remove tissue plugs from the hole punch using Q-tips or clean forceps and place both plugs directly into a vial (>3 ml) that has been prefilled with 95% ethyl alcohol. Label the vial with seal ID, etc. Upon returning to camp, fill out any other necessary information on the label and add a waterproof label to the vial (be sure it is small enough to be easily read from the outside should the external label fall off or become illegible). If necessary, top the vial off with additional alcohol so the vial is filled to the top with ethyl alcohol. Seal the vial with parafilm. If alcohol is not available, cover the tissue with salt and seal it up. Change the salt the following day, when most of the water has been driven out.

E. MOLTS, VIBRISSAE

1. Molt samples from known individuals should be collected for DNA analysis in the following order of priority:

a. Tear a sample (2cm x 2cm) and place in a 5 ml cryovial. Store in liquid nitrogen (LN).
b. Place an additional 2cm x 2cm sample in 95% ethyl alcohol in a 5 ml cryovial.
c. The remainder of the sample can then be stored in a whirlpak at room temperature.

2. Vibrissae can be stored in whirlpaks at room temperature.

F. PLACENTAS

Caution-there is potential for zoonotic disease transmission from seals to humans (see "Tagging and Handling" Protocol). TAKE PRECAUTIONS when handling all samples. Wearing disposable latex gloves, collect placentas found with or from an "aborted fetus" or perinatal pup death. Examine and collect these placenta according to the Hawaiian Monk Seal **Necropsy Protocol**. Also collect any fresh placentas observed in the following order (if not sure whether it's fresh, collect it):

1. Collect four 5 cm x 1 cm **full thickness** strips (extending through to include both the fetal and maternal sides) representative of normal and any abnormal portions of the placenta. Put 2 of each sample in 2 separate containers and fix in FM.

2. In addition to preserving sections in FM, freeze four 5 cm x 1 cm full thickness strips (extending through to include both fetal and maternal sides) representative of any abnormal portions of the placenta. **CAUTION: BE SURE samples are cut small enough to be removed from the LN dewar.**

G. TICKS

To collect ticks, look under objects (rocks, boards, buckets) near camp or bird colonies. The ticks like moist areas and can be found under the surface layer of the soil. Attempt to collect a minimum of 100 ticks (25/cryovial), label, and preserve in liquid nitrogen. If you see ticks on any animal that is being restrained (e.g., tagging a weaner), collect all ticks on the animal. Label ticks with seal ID of animal from which the ticks are collected. **Collect ticks as soon as possible-do not wait until the end of season!**

H. SKULLS

Collect all skulls found, even if seal ID and history are unknown. Preserve dry (**see Hawaiian Monk Seal Necropsy Protocol**)

I. NECROPSY SPECIMENS-see Hawaiian Monk Seal Necropsy Protocol

SPECIMEN COLLECTION-EPIDEMIOLOGY STUDIES ONLY

Specimens to be collected for epidemiology studies include:

Whole blood (in LTT, SST, GNNT, toxicology vials)
swabs (nasal, oral, eye, rectal, vaginal, prepucial)
blubber biopsies (for fatty acid analysis and toxicology)
fecal samples

1. BLOOD COLLECTION/PROCESSING: Code 1 ideal; Codes 2, 3, 4, 5 useless

BLOOD COLLECTION

Blood will be collected from all seals receiving a dose of diazepam and must be authorized by the attending veterinarian.

The following supplies are needed for blood collection:

betadine/ 70% ethyl alcohol squeeze bottles
scrub brush
1.5" and 18 x 3.5" spinal needles (3 per seal)
20 or 35 cc syringes (3 per seal)
extension set with "T" (2 per seal)
serum separator tubes- 6 minimum
3.0 mL Lavendar (LTT) - 1
9.0 mL GNTT-2
sharpie
cooler with blue ice pack.

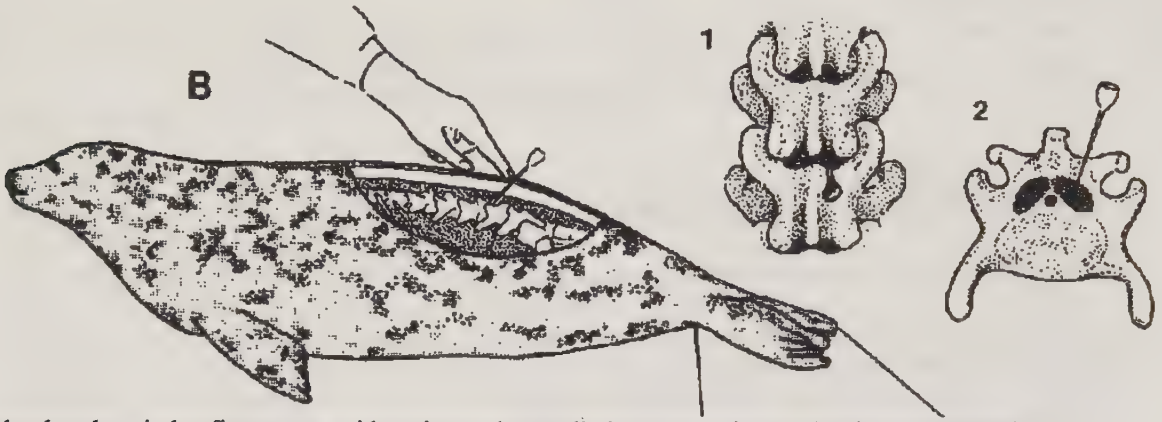
4' x 4" gauze pads or something to pad blood from contact with ice
styrofoam test tube holde (to keep SST upright in cooler)

All persons coming into direct contact with the seal or collecting specimens must wear gloves and other protective attire (see Clean protocol for handling seals in "Tagging and Handling" section of HMS manual).

Once the seal is restrained and positioned symmetrically in ventral recumbency (lying on its ventrum (or belly) with foreflippers tucked to the seal's sides), locate the vertebral column (spine) by using the thumb and another finger to straddle it; palpate the hips and move forward from the front most aspect of the hips approx. 2 vertebrae. Thoroughly clean this sampling site with betadine and scrub brush, then rinse with 70% ethyl alcohol. If the seal rolls on the sampling site, reclean it.

Before insertion, remove the needle from the plastic case, and if using a 3.5" needle, remove the stylet and plastic piece around hub. Hold needle from hub only **(i.e., do not contaminate the needle by touching the shaft)**. Protect the needle with your hand in a position so that the needle does not come into contact with anything during restraint.

Verbally inform restrainers that you are inserting the needle ("sticking") so they can be prepared in case the seal jumps.



Use the thumb or index finger as a guide to insert the needle between or just to the side of the dorsal spinous processes of the lumbar vertebrae (1) and into the bilaterally divided extradural vein (2) that overlies the spinal cord (Fig. 1). As the needle is inserted, feel it moving through skin, blubber, and muscle until you feel it pop through the membrane of the extradural sinus. You should now see blood rising to the hub of the needle. To move the needle once it is inserted, pull it up part way (until it is almost, but not completely, out) and then push it back in again. **Do not use a slicing motion to move the needle around because it may cause tissue and nerve damage.**

Initially, diazapam will be injected. Slow IV administration over a few seconds is preferred over a bolus injection. If the animal rolls or otherwise endangers itself with the needle, remove it as soon as possible. If the animal does not move significantly and then becomes sedate, leave the needle in place and this needle can be used to collect blood from the animal. If the needle is contaminated or removed for any reason, insert a new needle before collecting blood.

If you need to reinsert the needle do it at a different spot than the original location. The angle of the needle may vary from a 45 to 90 degree angle forwards or backwards from the dorsal surface of the animal. If the needle hits bone you know you are in the right area, so just reposition it. It is best to feel your way through the layers and stop before hitting bone, as this can cause the animal pain.

When the blood vessel is located (you will see blood coming out the top of the needle), attach the extension set with syringe to the needle while holding the needle in place with the opposite hand. Make sure to hold

the needle still and attach the extension set firmly. An assistant should connect the extension set to the syringe.

Collect 35 - 105 cc of whole blood by slowly drawing upward on syringe. Bubbles in syringe indicate your needle/extension set or extension set/syringe connections are not secure. This should be corrected as it may lead to hemolysis and poor filling. When finished collecting blood, disconnect syringe from the extension set, and **immediately remove needle from the seal.**

When filling tubes, remove caps (take care to keep inside top of cap from making contact with anything), tilt tubes and slowly push blood out of syringe and down side of tube to prevent lysis. First fill LTT and GNTT 3/4 full to allow for proper ratio of blood to anti coagulant, immediately replace cap, and mix the specimen by gently inverting the tube several times to thoroughly mix additive and blood. Fill scintillation or teflon vial (for toxicology) with 5-6 mL of whole blood. Then fill SST with remainder of blood. If special tubes for other studies are required, fill them accordingly.

During restraint, closely monitor respiratory rate, cardiac rate, eyes, and body movements and temperature of the seal being handled. Changes in temperature, breathing, and heart rate, unresponsive eyes, seizures, muscle weakness or spasms, and rigid/flacid flippers may indicate severe stress or shock. **If this happens, ABORT the procedure immediately. THE VETERINARIAN ON SITE WILL DIRECT EMERGENCY PROCEDURES AS NECESSARY.** Emergency procedures may include:

a) **moving away from the animal** to avoid any added stress
b) using emergency equipment to **establish an airway**
c) **cooling the seal** by drenching with water
d) **loading emergency drugs** (see below)
e) **taking careful notes**
f) **treating for emergency conditions** as necessary, i.e., capture myopathy, dehydration, cardiac arrest, vomiting,
wounds, seizures

Notify Marine Mammal Research Program Chief of the situation as soon as possible.

Emergency Drug Doses

DRUG	SEAL SIZE	DRAW	ADMINISTRATION
DOPRAM (Doxapram hydrochloride; 20 mg/ml)	Pup or juvenile	10 ml	Give 5 ml, slow IV Repeat if needed
DOPRAM (Doxapram hydrochloride; 20 mg/ml)	Subadult or adult	20 ml	Give 10 ml, slow IV Repeat if needed
ATROPINE sulfate (1/120 gr)	Pup or juvenile	1.5 - 3 ml	Give 1.5 ml IV or 3 ml IM
ATROPINE sulfate (1/120 gr)	Adult	2.5 - 4 ml	Give 1.5 ml IV or 3 ml IM
EPINEPHRINE (1:1000)	All sizes	5 ml	Give 1 ml/100-200 kg IM or IV, repeat prn
DEXAMETHASONE 4 mg/ml	All Sizes		Give 10 ml /50-100 kg IM or IV
FLUMAZENIL (Romazicon, 0.5 mg/5/ml)	Pup or juvenile	1 bottle (5 ml)	Give 2.5 ml slow IV (over 60 sec) Repeat in 2-3 min if needed
FLUMAZENIL (Romazicon, 0.5 mg/5/ml)	Subadult or adult	1 bottle (5 ml)	Give 2.5 ml slow IV (over 60-90 sec) Repeat in 2-3 min if needed

BLOOD PROCESSING

LTT, GNTT, and scintillation or teflon vials should be immediately chilled in cooler (avoid contact with ice to prevent cell lysis) or refrigerator. Blood in LTT is used for WBC counts. Blood in GNTT is used for collection of buffy coats and plasma. The GNTT and LTT (after hematology is completed) should be spun in the centrifuge and the plasma pipetted into cryovials. Buffy coats should be collected from GNTT and placed in cryovials. If sample collection is done in a remote area, total solids and PCV should be measured from plasma collected in LTT.

The SST tube(s) for collecting serum should sit in an upright position at room temperature or in the shade to clot for 15 - 20 minutes, not to exceed 1 hour. **Blood should be processed within 4 hours of collection.** Once the clot in the SST is retracted (i.e., visible separation), centrifuge tubes for 10 minutes at 2500 RPM or higher (using the appropriate speed and time according to your centrifuge). The blood will separate into 3 layers: serum on top (clear, yellowish); middle gel; bottom layer of red blood cells (dark red). Remove the serum using a clean pipet and place the serum in a 1.2 mL cryovials, label, and freeze in liquid nitrogen or ultrafreeze immediately. The first four 1.2 ml vials of serum (spec sub-#s A-D)will be kept for banking so be certain that they are full vials. Avoid using hemolyzed blood for these samples if possible. **If any vials of serum or plasma that are collected are not full, be sure to record the amount in the vial in the comments section of the "Monk Seal Specimen Collection Summary."**

Dispose of blood collection supplies. Place needles in sharps container and biohazardous waste in appropriately labeled bags.

2. SWAB COLLECTION

Use sterile dacron polyester fiber tipped swabs with plastic applicators. Swabs may be collected from sedated seals in the following order: rectum, nares, medial canthus of eye, lateral commisure of the mouth and genital orifice. Collect 2 swabs from each location, place in prelabeled cryovials, break off tips of swabs in sterile manner, and preserve in liquid nitrogen.

If swabs for bacteriology are indicated, they may also be collected at this time.

3. BLUBBER BIOPSY PROTOCOL: Code 1 ideal; Codes 2, 3, 4, 5 useless

Supplies needed:

betadine/alcohol in scrub bottles, scrub brush
Sterile surgical gloves
teflon or amber vial for tox biopsy
5.0 ml cryovial for Fatty acid biopsy
disposable scalpel #11
2 Accupunch biopsy punches
rat toothed forceps
scissors

This technique is to be performed by a veterinarian. Blubber samples will be taken from Hawaiian monk seals using a routine procedure requiring sedation (diazapam). Local anesthesia is an option the veterinarian may wish to consider, particularly in lightly sedated seals. The blubber biopsy is taken from the lateral aspect of the seals pelvic girdle, approximately 5-15 centimeters cranial to the wing of the ileum. The incisions should be made to allow proper fluid drainage. Before incisions are made, the area should be thoroughly cleaned with *povidone iodine solution and 70% ethanol (take precautions in windy situations to avoid getting ethanol in eyes of personnel). Wearing sterile surgical gloves, make two full thickness skin incisions using disposable, sterile #11 scalpel blade, through to the level of the blubber, 1-2 cm in length, and parallel to the long axis of the animal. introduction of the biopsy punch. Through each incision, insert a sterile 6mm biopsy punch. Take a blubber sample through the full depth of the blubber layer (2-5 cm). Use sterile or sanitized thumb forceps and/or scissors as necessary, without damaging or contaminating blubber

tissue. Do not allow gloves to contact tissue! The blubber sample from the first incision should be stored in a 5.0 ml cryovial and frozen in liquid nitrogen for fatty acid radioactive isotope analysis. A second blubber sample will be collected through the second incision for toxicology analysis. Follow the same technique but place samples in glass amber vials and frozen at -20° C as soon as possible. Blubber samples for toxicology are to be paired with 5-6mls of whole blood collected during the same restraint period that the blubber biopsies are collected. The blood sample should go directly from the withdrawing syringe into scintillation vials. The blood should be kept cold and frozen at -20° C as soon as possible. After samples have been collected, clean the incisions with *povidone iodine solution. Allow the wound to heal by second intention. Suturing is not desirable. When the biopsy is taken in this manner from harbor and grey seals, the incision has been found to heal almost completely within a week (S. J. Iverson & W. D. Bowen, pers. Comm., 1998). Over 200 blubber biopsies have been collected from LEvIS using this method, and no ill effect has been reported.

4. PVA-FIXED FECAL LOOP (PVA-FL) PROTOCOL: Code 1 ideal; Codes 2, 3, 4, 5 useless

Supplies needed:

Vials pre-filled with polyvinyl alcohol
fecal loop

The fecal loop should be inserted into the anus as far as possible. Using 12-15 ml screw cap vials prefilled with 5 ml of PVA fixative and premarked at the 7 ml fill level, add sufficient feces (~2 ml) from the loop to bring the liquid volume level to the fill mark. Screw on the cap tightly and mix thoroughly by shaking for 10+ cycles. Avoid collecting any extraneous materials such as sand, hair, or undigested food items.
GENERAL GUIDELINES FOR COLLECTION, PRESERVATION, STORAGE, AND HANDLING of specimens for diagnostics. Consultation with the laboratory on preferred procedures is required in all situations to obtain usable specimens. Tissues and specimens may be also preserved for other diagnostic activities, including **cytology, immunologic, and nutritional** studies (e.g., vitamin A, thiamine, radioactive isotopes) as required.

Diagnostic Activity	Type of Specimen	Preservation Method	Type of Container	General Observations
Histopathology	All tissues and lesions	10% buffered formalin	Leak-proof glass or plastic	Formalin ratio 10:1; room temperature
Toxicology	Organs, blood, blubber, ingesta	Refrigeration or freezing	Sterile glass or plastic, metal foil, teflon	Procedures change; need good records
Microbiology - virus, bacteria, fungi, rickettsia	Organs, tissues, swabs, lesions, body fluids, buffy coat	Refrigeration, freezing or ultrafreezing	Sterile cryovials, plastic or glass, special vials, and transport media	Avoid contamination; sampling varies with disease
Parasitology	Endoparasites*	AFA or AG solution	Glass or plastic	Room temperature
	Ectoparasites	AG solution or 10% buffered formalin	Glass or plastic	Room temperature
	Hemoparasites	Smears or whole	Glass slides or EDTA tubes	Room temperature Refrigerated

		blood		
Hematology and Plasma Analysis	Whole blood in lavender, green, and gray top vacutainers	Refrigeration until spinning, Liq. N_2 after pipetting	Pipetting of plasma into cryovials	Short storage, immediate processing
Serology	Blood	Refrigeration/ freeze (-20C) or ultrafreeze serum	Sterile cryovials	Handle gently to avoid red blood cell rupture
Genetics	Skin punch; buffy coat and red blood cells	Ethyl alcohol or salt; freezing or ultrafreezing	Vials; cryovials,	Long-term storage

PACKAGING AND SHIPMENT

IMPORTANT: Include original copy of completed Necropsy Report Form when sending necropsy specimens back to Honolulu. When sending ANY specimens, include a disk with the latest back-up of the specimen data base.

Notify Honolulu **several days in advance** before sending specimens back so that arrangements can be made to pick them up.

Update the transport information in the "Monk Seal Specimen Collection Summary" prior to sending any samples back to Honolulu (so that the data includes the shipping information).

All buckets and lids used for storing/transporting specimens should be labeled "SPECIMENS, NO FOOD" using a permanent marker.

Bucket lids should be labeled with specimen types (e.g., "SCATS," "NECROPSY TISSUES," "TISSUE PLUGS")

1. Pack all necropsy tissues and placentas together. Tissue plugs, molts, and PVA vials may be packed together. Scats and spews may be packed together, but do not **pack any other specimen types with them.** Be sure all specimens are **double bagged,** placed upright to avoid leaking, and be sure there is enough absorbent material packed around containers in case of leakage.

2. Be sure to secure nitrogen dewar lid with zip ties or duct tape. **Ensure that dewar is secured during transport so it cannot tip over.**

3. If sending specimens in a cooler, all individually packaged specimens may be placed together in one outer bag and then surrounded by packing materials and the proper coolant (i.e., blue ice, dry ice, etc.). Line the shipment container (e.g., bucket or cooler for frozen items) with a plastic bag and place absorbent packing material such as newspaper, paper towels, or gauze on the bottom and in between layers of sample containers; also fill any remaining space with packing material to secure samples. When shipping frozen items via plane or ship, put an ice pack in the cooler; if via ship freeze immediately once aboard the vessel.

4. Make sure lids on sample containers are secure (use Parafilm or tape if necessary), bags are well sealed, and containers are in an upright position.

5. Tissues that have been properly fixed may be transported without FM. For those specimens which are too large to be transported in secondary containers (e.g., GIT specimens in 5 gallon buckets), **FM MUST** be drained before transport. The used FM can be drained into carboys which should be clearly labeled with both the specimen number and bucket number from which it was derived. Field camp leaders are responsible for ensuring that the specimens **are refilled with the used FM upon return to Honolulu.**

All buckets containing specimens should be **clearly labeled** with the following:

Keep Upright
Sample type (i.e., scats, spews, necropsy tissue)
Preservation Method (e.g. 10% buffered formalin, polyvinyl alcohol)
Island/Year
Appropriate HazMat stickers
Caution - hazardous/infectious material (if applicable)

IV. REFERENCES

Dierauf, L.A. 1994. Pinniped forensic, necropsy, and tissue collection guide. U.S. Dept. of Commer., NOAA Tech Memo. NMFS-OPR-94-3, 80p.

Geraci, J.R., and V. J. Lounsbury. 1993. Marine Mammals Ashore: A field guide for strandings. Texas A&M University Sea Grant College Program, Galveston, Texas, 305 p.

Winchell, J.M. 1990. Field manual for phocid necropsies (specifically *Monachus schauinslandi).* U.S. Dept. of Commer., NOAA Tech. Memo. NMFS-SWFC-146, 55 p.

MONK SEAL SPECIMEN COLLECTION SUMMARY INSTRUCTIONS

For each specimen collected, log information in the "Monk Seal Specimen Collection Summary" found in Master Field Log. Enter data into the SPECIMEN database.

SPECIMEN

NUM Consecutive 4-digit numbers (0001 - 9999) of samples as they are collected, regardless of sample type. These numbers are unique for a given atoll/year. Each sample container should have its own specimen number and label.

SUB-NUM Complete only if a specimen is split. If the specimen is split, assign consecutive sub-numbers (aa - zz). A specimen can be split in the field or back in the lab. If you have a single specimen 0043, and you split it into two parts, then make one part 0043a and the other part 0043b. If 0043a is later split into two parts, make one part 0043aa and the other 0043ab. If 0043a has been split so that you have 0043aa, 0043ab, and 0043ac, and you want to split 0043aa, then leave one of the new split parts as 0043aa and make the other part the next available sub-number (i.e., 0043ad). Each container must be labeled, and must have a unique number/sub-number.

TYPE Type of specimen collected.

BB = blubber biopsy, collected from live animal
BC = blood cells (remainder in tube after removing plasma or serum)
BL = blood (as for virology scint. vials)
BU = buffy coat
BS = blood smear
ES = eye swab
FL = fecal loop
GI = gastrointestinal tract section **taken anywhere from pharynx to anus**
MO = molt
NS = nasal swab
NT = necropsy tissue (tissues from all organs, includes necropsy blubber sample)
PC = placenta
PL = the 'pluck': heart, trachea, lungs and associated glands
PS = plasma
RS = rectal swab
RT = whole reproductive tract of female or male
SC = scats
SE = serum (collected in serum separator tube for serology)
SK = skeletal (bones or teeth)
SP = spew
ST = stomach contents
TP = tissue plug
VS = vaginal swab
WB = whole blood
WC = whole carcass
WH = whole heart
ON = other necropsy (non-tissue samples, e.g., bile, urine, aqueous humor)
OS = other swab
OT = other (indicate type in notes section on back of form)
NO = none

SUBTYPE Subtype of specimen collected (i.e., parasite from a spew). Distinguish necropsy tissue subtypes (*) for frozen toxicological samples.

AD = adrenal (*)
BC = blubber chunk (necropsy tissue)
BM = bacteriology medium
BP = blubber biopsy plug (necropsy tissue)
BR = brain (*)
BT = blue topped tube (BTT)
DN = DNA analysis
GI = sample from gastrointestinal tract (*)
GN = green topped tube (GNTT)
GT = grey topped tube (GTT)
KI = kidney (*)
LI = liver (*)
LT = lavender topped tube (LTT)
LU = lung (*)
NP = not processed/intact (scats and spews)
OL = otolith
PA = parasites
RT = red topped tube (RTT)
SP = spleen (*)
SS = serum separator tube (red/grey topped; SST)
VM = virology medium

SEAL ID Record the 4-digit permanent ID number of seal if known.

TEMP ID Record the temporary ID number (or bleach number) of seal if known.

SIZE

P0 = Fetus (aborted, clearly pre-term pup)
P = Nursing pup
- P1 = Nursing pup, wrinkles
- P2 = Nursing pup, no wrinkles
- P3 = Nursing pup, blimp, black
- P4 = Nursing pup, molting
- PS = Nursing pup, molted

PW = Prematurely weaned/undersized weaned pup (weaned ≤ 2 wks ago and < 90cm girth)
W = Weaned pup
I = Immature
J = Juvenile
- J1 = Juvenile I
- J2 = Juvenile II

S = Subadult
- S3 = Subadult III
- S4 = Subadult IV

A = Adult
U = seal of unknown size

SEX M = Male, F = Female, U = Unknown

SURVIVAL FACTOR NUM Survival factor number.

COLLECT DATE Date sample is collected (in MMDD format).

ISLET Two-digit numerical code for the islet from which the sample is collected.

PRESERVATION METHOD at **COLLECTION,** during **TRANSPORT,** and on **ARRIVAL** in Honolulu

AA = absolute ethyl alcohol (98-100%)
AF = AFA: 50ml of 95% ethyl alcohol (not denatured), 5ml of acetic acid, and 45ml of 10% formalin (or 10 ml formaldehyde and 35ml of water)
AG = 95mm of 70% ethyl alcohol (not denatured) and 5ml glycerin
AL = 70% ethyl alcohol
CB = chloroform, BHT mix for blubber biopsies
DM = DMSO
DR = dry
FR = frozen
FM = 10% buffered formalin (neutral buffered formalin=NBF)
GA = 20% glycerin and 80% ethyl alcohol
LN = liquid nitrogen
PV = polyvinyl alcohol
RE = refrigerate
RT = room temperature
UF = ultrafreeze
OT = other (indicate method in notes section on back of form, use for whole scat w/detergent)
NO = none (sample was discarded and not preserved)

TRANSPORT MEANS Method of transportation used to ship sample to Honolulu.

CV = charter vessel (ship)
CP = charter plane
MP = military plane
OS = Oscar Sette
OT = other (indicate method in notes section)

DATE SENT Date sample sent from the field.

PROJECT: Code what project the specimens were collected for.

UM = UME
EP = epidemiology
PA = population assessment
PS = pup survival
ST = satellite tagging
CC = critter cam
OT = other

NOTES Blank = no handwritten notes for a sample.
1 = handwritten notes for a sample are on the back of form, labeled by sample number. Enter these into the Notes field of the database (do not exceed 250 characters).
2 = sample collected, analyzed, and discarded.

INIT Three initials of collector. If no middle initial, use the first and last block.

The following fields will be filled out later in Honolulu:

DATE ARRIVED Date sample arrived in Honolulu.

DIAGNOSTICS

LAB Location sample sent for analysis. Code as follows:

- AF = Armed Forces Institute of Pathology (AFIP)
- CA = University of California-Davis
- CO = Colorado State University-Fort Collins
- CV = CVD/IDDEXX
- GL = Gwen Lowe
- MK = Michael Kliks
- NW = NWHRC-Honolulu Station
- NY = Cornell University-Ithaca, New York
- OA = Oklahoma Animal Disease Diagnostic Laboratory
- OS = Oregon State University
- SC = NMFS- Charleston, South Carolina
- UH = University of Hawaii
- US = USDA
- WA = NMFS- Seattle, Washington
- WD = Washington Animal Disease Diagnostic Lab
- YL = Ron Lu (UH)
- OT = other

DATE SENT Date sample sent for evaluation.

EVALUATION Code for the diagnostic tests performed.

- BA = Bacteriology
- BP = Blood profile
- CP = Chemistry profile
- CY = Cytology
- EM = Electron microscopy
- EP = Endocrinology profile
- GE = Genetics
- HP = Histopathology
- IM = Immunology
- MY = Mycology
- NP = Nutritional profile
- PA = Parasitology
- PR = Prey analysis
- SE = Serology
- SM = Blood smears
- TX = Toxicology
- UA = Urine analysis
- VI = Virology
- NE = Necropsy
- OT = Other

RESULTS Results of diagnostic tests. Codes not yet available.

DATE TESTED Date of laboratory procedure.

SPECIMEN STORAGE:

FACILITY

HL= Honolulu Lab (Dole Street)

KR= Kewalo Research Facility

LOCATION

A2= Annex 2

DH= Diamond Head Lab

RE= Regular Freezer

UF=Ultra freezer

SHELF

BIN/BOX

CELL

APPENDIX B:

Hawaiian Monk Seal Necropsy Protocol

28 Jan 2003/RCB

HAWAIIAN MONK SEAL NECROPSY PROTOCOL

NOTE: Read the "Field Manual for Phocid Necropsies (FMPN) (specifically *Monachus schauinslandi)"* and this Necropsy Protocol carefully and several times before performing necropsies.

Necropsy or post-mortem examination of monk seals is a basic tool for the investigation of disease and monitoring the health of wild populations. It can be defined as the systematic examination of the whole body, organs, and tissues. **Whenever possible, necropsies should be performed by a trained veterinary pathologist** experienced in recognizing and interpreting lesions and abnormalities.

General Considerations

A necropsy has the most scientific value when it is carefully documented. The use of this Necropsy Protocol will assist in the documentation and standardization of information which may be valuable in determining morbidity and mortality factors within individual seals as well as the population.

Preventing Disease Transmission

Avoid direct contact with dead seals to prevent transmission of infectious diseases that are pathogenic to humans. Persons performing the necropsy must:

1. Wear protective gear, including latex or plastic (untorn) gloves, mask, coveralls/apron and rubber boots.
2. Cover your surface wounds with protective dressings before putting on gloves.
3. Collect frozen specimens only at locations where freezing is possible. **DO NOT STORE SPECIMENS IN FREEZERS/REFRIGERATORS USED FOR HUMAN FOOD.**
4. When the necropsy is completed, wash skin thoroughly with antibacterial soap and wash/sanitize or dispose of all contaminated clothing in a container clearly marked "Biohazardous waste."
5. If possible, pull the carcass to high ground (so it won't wash into the water) and bury to avoid attracting predators and disease transmission.
6. **Scalpel blades must be disposed of in sharps containers.** Disinfect instruments with a 50% chlorine bleach solution and thoroughly rinse with fresh water; or wash with soap and fresh water and then rinse with 70% ethyl alcohol. Keep all necropsy supplies clean and dry.
7. Seek medical attention for bites, cuts and other injuries and notify physician of their source.

General considerations in performing a necropsy include:

1. A complete and careful record must be kept of what is observed during the necropsy.
2. First obtain morphometric data, then external samples.
3. The order of the Necropsy Report Form follows the sequence of general dissection and examination. See sample collection and preservation sheet.
4. Tissues and organs must be examined in a systematic manner. The precise method used for a necropsy is less important than establishing a routine in which each body system is examined fully.
5. Once the carcass is opened, take tissue specimens for virology, bacteriology, and toxicology (only if a freezer and/or liquid nitrogen is available), then sample for histopathology and parasitology.
6. The objective is to recognize abnormalities; this requires the ability to distinguish between normal and abnormal tissues.
7. Samples of **normal and abnormal** tissues should be collected for laboratory analyses.
8. With an "aborted fetus," perinatal death, or newborn, perform necropsy according to "Fetus" section of necropsy form.

The ability to obtain reliable data from necropsies depends on the following:

1. Condition and location of the specimens.
2. Adherence to detailed protocols.
3. Number of seals to be necropsied throughout a field season.
4. Amount of YOUR time available to perform a thorough necropsy.
5. Care in sample preservation and labeling of specimens.
6. Care in shipping and storing specimens.

Decomposed carcasses are unsuitable for histopathology but are useful for describing gross lesions. Brain, intestines, liver and other enzyme-rich organs are the first to deteriorate. To date, the etiology of the eye condition of the captive seals remains unknown, therefore you should collect **eyes first**. Also, if the carcass is a code 1 (see page 8 for carcass condition codes) and the necropsy is being performed less than 1 hour after death has occurred, the **brain** should be collected **second**. Take samples from all organs listed, even those that appear normal. Tissue specimens must be sufficiently thin (less than 1 cm thick) to allow fixation with formalin except for brain and lung. Make parallel cuts (½ cm in thickness) in formalin fixed samples to allow preservation. Samples preserved in formalin should be fixed in 10% buffered formalin with 10 units volume of formalin to 1 unit tissue volume. Throughout the necropsy, refer to the FMPN.

Specimens for toxicology from **live** animals may be limited to blood and biopsies of skin and blubber (see Blubber Biopsy Technique). When sampling for toxicology, it is important to use standardized sampling procedures so that even when low levels of contaminants are present, differences may be attributed to biological processes and contaminant exposure and not to variation in the collection process. Tissues from dead monk seals must be collected **less than 24 hours** from time of death. As soon as possible, **freeze at lowest temperature** available.

1. Toxicological analyses may be performed for heavy metals, organochlorides, selenium, and dioxin. Tissue samples will be taken in duplicate (2 of each, except when noted).

2. See FMPN for collection techniques. Use a new, sterile blade for each organ collected. Any stainless steel instruments used in contact with tissues should be cleaned with distilled water and rinsed with isopropyl alcohol before using if possible. Each specimen should be rinsed with distilled water (if possible), wrapped in aluminum foil (with dull side touching specimen), placed in whirlpaks or ziplocks, then frozen.

3. **Collect the following samples by special request only**: Biotoxin (ciguatera poisoning) and/or hydrocarbons (oil spills) specimens may be collected at selected sites. The specimens, also collected in duplicate, include:
   a. Blubber: One, 100 gram sample, rinse with distilled water, wrap in Teflon sheet, place in whirlpak or ziplock.
   b. Liver: One, 100 gram samples, rinse with distilled water, wrap in Teflon sheet, place in whirlpak or ziplock.

4. Because analyses may involve detecting extremely small amounts of contaminants, **care must be taken in the collection and processing of these tissues.** Avoid salt water, tobacco smoke, bug sprays, and other aerosolized foreign materials during collection.

5. **Tissues should be collected as rapidly as possible** after opening body cavity to prevent contamination and deterioration.

TISSUES FOR MICROBIOLOGY: Code 1 ideal; Codes 2, 3 limited; Codes 4, 5 useless

Collect the following by special request only: For special studies, you may be required to collect additional specimens from monk seals.

Specimen collection for bacteriology and virology is determined primarily by the nature of gross pathologic lesions. Samples should be taken aseptically, from external surfaces, body cavities,and internal organs as soon as they are exposed. Place swabs in respective transport media and refrigerate at 4° C or place on blue ice immediately and freeze upon arrival to laboratory or field camp. If cryovials are available, ultrafreeze the swabs with tissue samples in liquid nitrogen. Samples for microbiology are worth the time and effort only when tissues are in suitable condition. With an "aborted fetus," perinatal death, or newborn, collect specimens according to "Fetus" section of Necropsy Form for freezing and later microbial analysis.

INSTRUCTIONS

A. Before conducting a field necropsy, become familiar with the **"Field Manual for Phocid Necropsies (specifically *Monachus schauinslandi)"*** for explanations of external and internal examination. Refer to this manual during the necropsy. Also become familiar with the protocol BEFORE performing necropsies.

B. Read the **"Specimen Collection Protocol"** for instructions on collection, preservation, packaging, and shipment techniques of samples collected

C. Use the Hawaiian Monk Seal Necropsy Report Form for **all** seals found dead during the field season, whether or not a necropsy was performed. Record "N/A" for any sections that are not applicable, and state what organs/tissues were not examined. At a minimum, identify the appropriate descriptors for each organ examined and sample all organs.

D. If the seal has flipper tags, note the condition of the tags on the census and tag condition forms. Recover flipper tags, place in a whirlpak bag and label the bag with animal ID, island, date, and survival factor number. Also check for PIT tags with the PIT tag reader. Even if PIT tags were not found during a scan, please indicate that a scan was completed. Also note where on the body the scan was performed. Record this information on the census sheet as well. If the ID of the seal is unknown or questionable, document any specific external lesions, abnormalities, or scar patterns. For seals that appear to have been mobbed, examine, describe, and photograph the mobbing injuries, anogenital area, scars, and other distinguishing characteristics such as injuries and external lesions. Record all photographs on census form and on the Necropsy Report Form as requested.

> **TAKE INTERNAL PHOTOGRAPHS ONLY WHEN UNUSUAL CONDITIONS ARE NOTED OR IF YOU ARE UNSURE IF IT IS UNUSUAL.** If unusual conditions are noted, include a size reference (e.g., ruler) and label with seal ID, survival factor number, date, and island. Take two photographs, one with the organ *in situ* (in its anatomical position/location) in the body and one with the organ removed from the body and placed on a solid white or light blue surface.

E. Measure axillary girth, dorsal length, and ventral length. Refer to the Necropsy Report Form for seal carcass measurements requested. **Record measurements on both the TAGGING/HANDLING CARD and the Necropsy Report Form.**

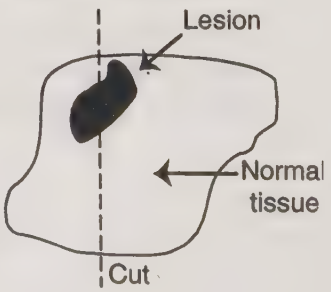
F. Carcass evaluation. A carcass condition code system has been developed based on the state of decomposition of a monk seal carcass (see page 8). Carcass condition is influenced by many factors including disease, body temperature, and environmental temperature. ***Rigor mortis*** (stiffening of the body following death) may serve as an indicator of carcass evaluation. It can occur within hours in warm weather, but is extremely variable. *Rigor mortis* indicates that a carcass may be in good condition (see Code 2 in Necropsy Report Form).

G. Assume more is better when describing and recording information. The rule here is if in doubt, write it down. If unsure whether something is abnormal, state this and succinctly describe. Descriptions should be clear, concise, and without personal interpretation. Appropriate tissue preservation along with YOUR precise description of findings may allow the identification of causes of death in the population.

H. Identify the appropriate descriptors for each organ examined. The descriptions provided herein are NOT an exhaustive list of terms, but rather a list for your reference. Describe surface, consistency, color, and cut surface of both normal tissues and abnormalities or lesions.

DESCRIPTORS of ORGANS AND LESIONS

Surface:	Smooth, rough, shiny, dull, thickened, wrinkled.
Consistency:	Firm, soft, flabby, dry, wet, fluid-filled, sharp-edged, friable (easily pulverized or crumbled).
Color:	Translucent, opaque, green, yellow, brown, pink, red, nutmeg (normal pattern of liver), etc. Also comment how color is spread through tissue, if it is homogenous or speckled (i.e., pink with specks of red).
Cut surface:	Slice organ several times appropriately and spread apart to look at internal surface. Be sure to describe color of the cut surface. Descriptors include swollen, bulging, shiny, dull, eroded, glistening, scaly, pitted, oozing.
Size:	Record in metric system (mm, cm), measure length, width and depth, or diameter of the lesion. Enlarged (hypertrophied), small (atrophied), normal size.
Shape:	Square, rectangular, triangular, oval, round, cuboidal, spherical, discoid, rhomboid, tear- shaped, wedge-shaped, spindle-shaped, irregular, long, slender, indented, narrow, lace-like, tortuous, branching, speckled (miliary), flat, raised, depressed, shrunken, papillary, cauliflower-like.
Distribution:	Single discrete lesion (focal), multiple lesions in one location (multifocal), or multiple lesions scattered diffusely throughout the organ or body cavity (diffuse); locally extensive, random, even.
Location:	Surface, capsule, wall, dorsal or ventral, caudal or cranial, anterior or posterior, medial or lateral, proximal or distal, internal or external, full or partial thickness of a wall of an organ.
Color:	Additional descriptors may include bright, pale, streaked, blotchy, dark, Blanched, mottled yellow with brown specks, brick red, Christmas red, yellow with white streaks yellowish white, blotchy red brown, creamy, off-white, transparent, opaque. Use simple colors, do not get complicated.
Fluid:	Clear, cloudy, turbid, thick, thin, bloody, muciod, exudate, dark, tarry.
Consistency:	Spongy, granular, gel-like, firm, soft, hard, rock-hard, dense, creamy, buttery, brittle lumpy, velvety, warty, tenacious, gritty.
Texture on cut surface:	Bulging, engorged, granular, nodular, pitted, oozing
Odor:	None, sweet, sour, rancid, ammonia-like, putrid, fruity, petroleum-like



I. **COLLECT ALL LESIONS**. Describe and sample areas that appear to stand out in marked contrast to the main body of tissue. Include a description of the margins between the normal and abnormal tissue

i.e., sharp line versus vague and gradual, circumscribed, encapsulated.
When collecting abnormal tissues for histopathology, be sure to include the margin between the abnormal and adjacent normal tissue and accurately describe abnormalities (including precise location).

J. Review the completed Necropsy Report Form and file in the Master Field Log. Review census form for sighting of animal as dead, and include survival factor number, photos taken, tag condition, and tags recovered. Make sure that the tagging/handling card, scar card, and tag condition drawing form are complete.

K. Record specimens collected on the Specimen Collection Summary and assign specimen numbers as outlined in the Specimen Collection Protocol. **The 2 jars of tissue sets for histopathology (HP) should have the same specimen number but a different sub-number.** Make sure to check the boxes next to the appropriate specimens as collected on the necropsy report form.

L. Necropsy Report Forms, scar cards, tagging/handling cards, survival factor forms, and photos should be returned to the NMFS Honolulu Laboratory.

M. Clean necropsy tools (you may also need to spray them with WD-40 or LPS) and restock necropsy kit so that it is ready for the next necropsy.

EQUIPMENT AND SUPPLIES FOR A SINGLE NECROPSY

1. Clipboard, metal
2. Necropsy Table (Checklist)
3. Necropsy Report Form(s)
4. Necropsy Manual
5. Scarcard
6. Census Form
7. Tag/handling card
8. Survival factor form
9. Self-sticking pre-printed specimen labels (3 sheets)
10. Waterproof labels ("rite-in-rain" paper, pre-cut) (3 sheets)
11. Pencils (2)
12. Ball-point pens (optional) (2)
13. Permanent markers (sharpies) (2)
14. Ruler (metric)
15. Metal tape measure (metric)
16. Tape measure, flexible (metric)
17. Surgical scissors (2: straight and curved)
18. Rubber gloves
    chemical resistant - 2 pr. latex
    heavy duty - 6 pr. latex exam gloves - 15 pr
19. Scalpel handles (4: 2 #8, 2 #6)
20. Scalpel blades (30)
21. Blubber biopsy punch, 6 mm (2)
22. Forceps (2 pr.)
23. Hemostats (1)
24. Dacron Swabs *(5)*
25. Microscope slides
26. Slide holder for microscope slides
27. Small vials (—2 x ½") - 6 ea.
28. Med. vials (—3' x 2) - 6 ca.
29. Whirlpak bags (20 ea.)
30. Ziploc bags (large)
31. Paper towels
32. Antibacterial soap/Hand sanitizer
33. Sharps container
34. Needles, 1 ½', 18 g *(5)*
35. Syringes, 20 cc or 35 cc *(5)*
36. Blood tubes, PT, TT (4)
37. Biohazard bag (2)
38. Camera with colored slide film and/or Digital camera
39. Shovels
40. Jars and buckets partially filled with 10% NB Formalin
    a. 2 large jars for duplicate sets of histopathology tissue samples
    b. 1 bucket for entire female reproductive tract
    c. 1 bucket for esophagus
    d. 1 5gal. bucket for entire gastrointestinal tract from beginning of stomach to anus
    e. 2 jars for eyes
    f. 2 jars for brain
41. Hacksaw

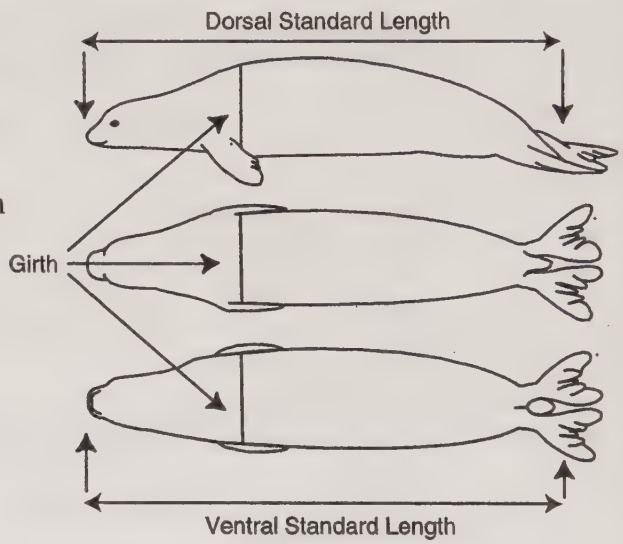
42. Axe
43. Cryovials (20)
44. String to tie off beginning of stomach, etc. (1 roll)
45. Cutting board (not on spc. coil. list)
46. Disposable aprons (3)
47. Bone shears
48. Archival tag labels for boxes
49. Dissecting tray
50. White or light blue plastic sheet for photographic background
51. 85% saline for relaxing parasites
52. 10% NB Formalin
53. Ethyl Alcohol *95%*
54. Isopropyl Alcohol 70%
55. Glycerol
56. Teflon container (3)
57. Aluminum foil (1 roll)
58. Knives
59. Blank notecard

17 Jan/RBC

HAWAIIAN MONK SEAL NECROPSY REPORT FORM

NECROPSY #_____(Assign Necropsy # in order of necropsies performed (only assign # if necropsy is actually performed). For example: if this was the second necropsy performed during the 2003 season, the Necropsy # would be 2).

BACKGROUND INFORMATION:



Seal ID No _____
Survival Factor Number _____
Temporary ID Nos_____
Island/atoll _____ Islet in atoll _____
Size/sex _____Age (if known)
Sector _____ Beach Position _____

Collect/record tag condition
Temple tags _____
Date/time found dead
_____
PIT tags read: Left _____
Date/time last seen alive
_____
Right_____

Body measurements (also record on Tag/Hand Card):
Axillary girth_____ cm
Dorsal standard length _____ cm
Ventral standard length _____ cm
Total body mass _____ kg
Measurer _____

Died/euthanized/other (explain) _____
_____
_____
_____

Photographs taken? (if yes, add roll #s to Census Form) _____

_____

DATE/TIME OF NECROPSY_____**RECORDER:** _____

ATTENDING DVM/ PERSON(S) PERFORMING NECROPSY:_____

CLINICAL HISTORY:

Reproductive status _____ Prior injuries/marks _____

Last event seen, etc.

_____

_____

_____

PROBABLE CAUSE OF DEATH:

Based on gross findings (field diagnosis) _____

Based on histopathology: _____

ENVIRONMENTAL CONDITIONS (around the approximate time of death):

Environmental factors (extreme weather conditions, i.e., dry/hot, storms, precipitation, temperature changes or other changes that may contribute to stress)_____

_____

Ambient Temperature _____ Cloud Cover _____

Water Temperature _____ Rainfall _____

Wind Speed _____

Wind Direction _____

GENERAL CONDITION:

Carcass Condition Code

Code 1: **just died**

Code 2: **fresh**/carcass in good condition (rigor mortis, fresh smell, normal appearance, minimal drying of skin and mucous membranes, eyes clear, carcass not bloated, muscles and blubber firm, viscera intact and well-defined, guts with no gas)

Code 3: **fair**/decomposed (carcass and organs intact, bloating, skin sloughing, mild odor, eyes sunken, dried mucous membranes, friable viscera, blubber oily, muscles soft but still intact, gut dilated with gas)

Code 4: **poor**/advanced decomposition (carcass may be intact but collapsed, skin sloughing, strong odor, blubber soft with pockets of gas, liquified organs, blood thin and black, viscera friable difficult to dissect and easily torn, gut filled with gas)

Code 5: **mummified**/skeletal remains (skin draped around bones, remaining tissues desiccated)

Gas formation (run hands firmly over body to feel for gas): Y/ N

Location:_____

Maggots: Y/ N Maggot location:_____

Examine all outside surfaces and note all anomalies. Photograph scars and other distinguishing Characteristics (see Necropsy Instructions section D):

Nutritional state (fat/overweight; normal/average; thin/poor; starving/emaciated): _____

_____

Hydration state (good; slightly dehydrated; moderately dehydrated; severely dehydrated; unknown): _____

Pelage/fur coat (normal; fur loss; oil; molting; scruffy; parasites): _____

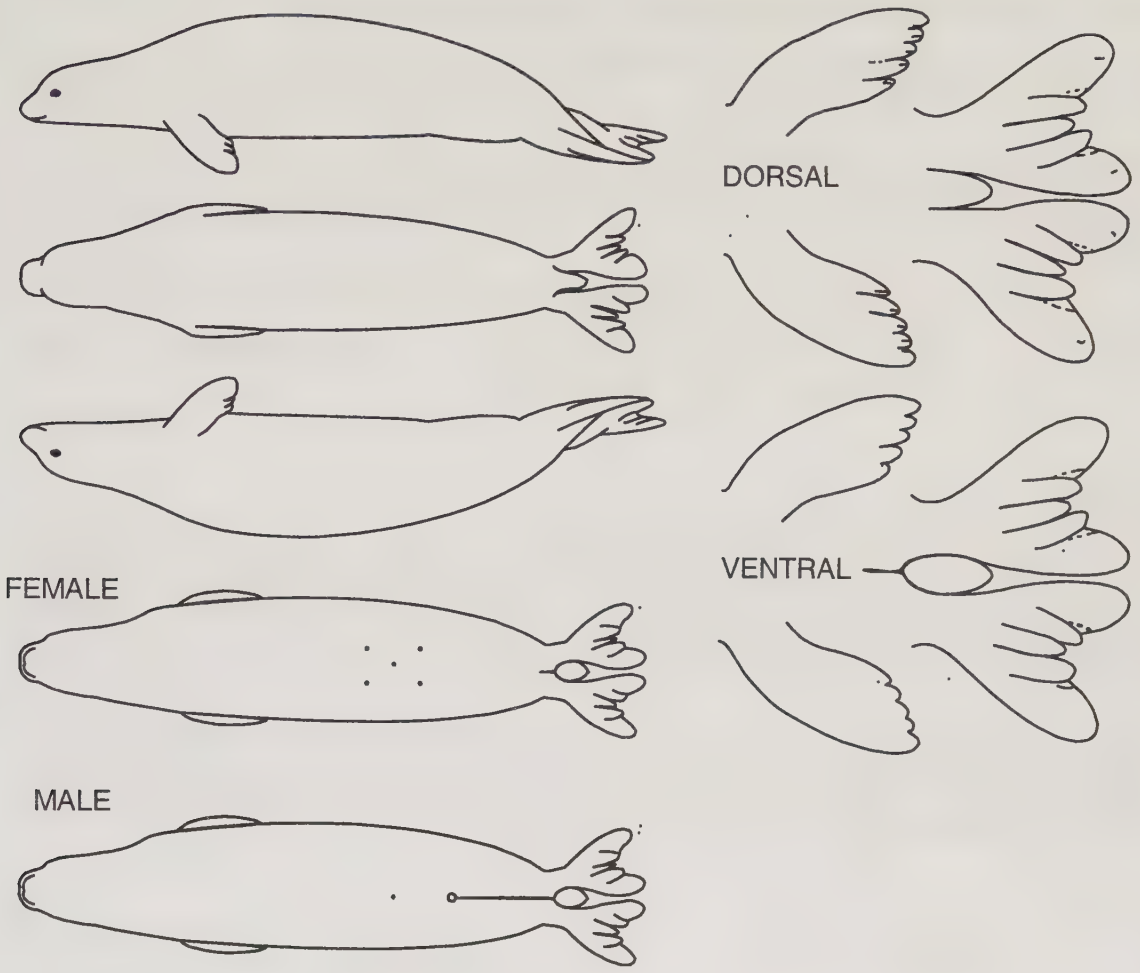
Skin (normal; cracking; bleeding; dried; moist; smooth; rough; wounds; infection; masses; parasites; scars):_____

Nails (normal; present; absent; torn; bleeding; cracked; crushed): _____

Vibrissae/whiskers (normal; present; absent; torn):_____

Peripheral lymph nodes (palpable; obvious; unnoticeable; other): _____

Abnormalities and markings (include bruises, wounds, contusions, old scars, condition of and tears of skin, external parasites). Copy scar card or make drawings as appropriate.



DESCRIBE Conditions of All Organs and Tissues and Any Lesions (Use The Descriptors Provided)!

EXTERNAL EXAMINATION

Eyes (Describe):

Eyes should be collected first. Remove both eyes with eyelids and lacrimal gland.

Eye, right - fix in formalin. Make a 2-3 cm cut in the globe along the interface of the sclera (white part) and the part normally in the eye socket.

Eye, left - ultrafreeze if liquid nitrogen available; otherwise fix in formalin

Attach sterile 18 g x 1.5" needle to sterile syringe and insert needle to collect aqueous humour from both eyes.

Sp #_____ A Right Eye with Eyelid ☐ (FM) Sp #_____ Aqueous Humour ☐ (LN)
B Left Eye with Eyelid ☐ (LN OR FM)

Brain:

Brain should be collected second only if the carcass condition is code 1 and it has been less than 1 hour since death has occurred. Expose the brain on fresh specimens only (Codes 1,2). Remove the top of the skull and cut away the soft tissues over the cranial vault. The procedure requires a good set of bone cutters and cleaver and/or ax/hatchet. Clip around the sides and cut dura mater (thin fibrous covering) attached to bone and then cut the *tentorium cerebellum* (thick connective tissue roof over cerebellum) with bone forceps and remove the skull cap.

Remove INTACT. Handle gently and use sufficient formalin. Split right and left halves of brain using a new scalpel blade. Slice right half into 3 sections from front to back and fix for HP; slice left half into 1 cm wide sections from side to side (medial to lateral) and fix for HIP. Collect four 25 gm (1x2x2cm) sections from the left and right lobes and wrap in foil for toxicology analysis.

Sp # _____ A Left Brain ☐ (FM) C Left Brain 25 gm ☐ (LN) G Right Brain 25gm ☐ (LN)
B Right Brain ☐ (FM) D Left Brain 25 gm ☐ (LN) H Right Brain 25 gm ☐ (LN)
E Left Brain 25 gm ☐ (LN) I Right Brain 25 gm ☐ (LN)
F Left Brain 25 gm ☐ (LN) J Right Brain 25 gm ☐ (LN)

Skull:

If the brain is not removed, save entire skull including mandible (flense and let sit outdoors in a container to be reduced by bugs). If the brain is removed, remove mandible (lower jaw) and all teeth within it and preserve as above.

Sp #_____ Entire Skull ☐ **OR** Sp #_____ Mandible ☐

Mouth/Teeth (Describe conditions of mouth and teeth):

Dental formula (Incisors (front teeth before canines) 2/2, Canines 1/1, Post-Canines (all teeth behind canines) 5/5 each side):
I _____, C_____, PC ____ each side

Describe nares:

Insert sterile swab into nares, then place in cryovial.
Sp #_____Nasal Swab ☐ (LN)

Look for nasal mites, if observed, collect with sterile swab and place in vial filled with AG/FM.
Sp #_____ Nasal Mites ☐ (AG/FM)

Describe penile opening/ vulva (circle appropriate orifice):

Sp #_____Genital ☐ (LN)

Describe anus:

Blubber Biopsy:
The blubber biopsy is taken from the lateral aspect of the seal's pelvic girdle, approximately 5-15 centimeters cranial to the wing of the ileum. Gloves, scalpel/knife and sterile biopsy punch are to be used. One full thickness skin incision, through to the level of the blubber, 1-2 cm in length, and parallel to the long axis of the animal is made before the introduction of the biopsy punch.

Through this incision, a sterile 6 mm biopsy punch is inserted, and a blubber sample is taken through the full depth of the blubber layer (2-5 cm). Use sterile or sanitized thumb forceps and/or scissors as necessary without damaging or contaminating blubber tissue. No glove contact! This blubber sample will be stored in a cryovial and frozen in liquid nitrogen for fatty acid radioactive isotope analysis.

Sp#_____ A Blubber Biopsy ☐ (LN)
Sp# _____ B Blubber Biopsy ☐ (LN)

INTERNAL EXAMINATION

Do the internal examine in 4 phases: integument, head, thorax, and abdomen. Open each part separately to help prevent contamination and drying out of tissues.

Blubber (measure at sternum or chest between the front flippers).
Sternal blubber thickness: _____ (mm)

1. INTEGUMENT

Hawaiian monk seal dermis and epidermis are fairly thick and dense. The deeper layers are firm and white. Immediately beneath this the blubber layer begins and for all practical purposes continues until the muscle begins.

If a carcass is code 1 or 2, two sets of histopathology samples from the organs listed in the Necropsy Protocol should be collected. Both sets of HP samples will have the same specimen number, but two different sub-numbers as follows:

Tissue Set Jar #1 = Sp #_____ A

Tissue Set Jar #2 = Sp #_____ B

Vibrissae:
When collecting vibrissae, include a 2 cm x 2 cm x 1 cm thick piece of tissue attached to vibrissae.

Tissue Set Jar #1
Left Vibrissae ☐ (FM)

Tissue Set Jar #2
Right Vibrissae ☐ (FM)

Flippers:
Take small pieces of each flipper with nail and fix in formalin, as 2 sets. Also take a tissue plug from rear flipper and fix in 95% ethyl alcohol for genetics.

Tissue Set Jar #1
Front Flipper ☐ (FM)
Rear Flipper ☐ (FM)

Tissue Set Jar #2
Front Flipper ☐ (FM)
Rear Flipper ☐ (FM)

Sp #_____ Flipper (Tissue Plug for genetics) ☐ (AA)

2. HEAD

Remove head from body and examine mouth, tongue, and collect lymphoid tissues and salivary glands.

Lymph Nodes
Submandibular with salivary glands (Describe):

Tonsil (Describe):

Tongue (Describe):

Tissue Set Jar #1
Submandibular/salivary gland ☐ (FM)
Tonsil ☐ (FM)
Tongue ☐ (FM)

Tissue Set Jar #2
Submandibular/salivary gland ☐ (FM)
Tonsil ☐ (FM)
Tongue ☐ (FM)

Unless carcass is a code 1 and the brain has already been removed, place head aside in a cool area and remove the brain last.

3. THORAX

Take 2 specimens including **skin, subcutaneous tissue, and muscle** for HP. Also collect 8 specimens of blubber without skin for toxicology (see FMPN p. 5) and 2 additional specimens of muscle **only** if animal was euthanized.

Skin (Describe):

Tissue Set Jar #1
Skin ☐ (FM)

Tissue Set Jar #2
Skin ☐ (FM)

Blubber (Describe):

Tissue Set Jar #1
Blubber ☐ (FM)

Sp# _____
A Blubber 25 gm ☐ (LN)
B Blubber 25 gm ☐ (LN)
C Blubber 25 gm ☐ (LN)
D Blubber 25 gm ☐

Tissue Set Jar #2
Blubber ☐ (FM)

(LN)
E Blubber 25 gm ☐ (LN)
F Blubber 25 gm ☐ (LN)
G Blubber 25 gm ☐ (LN)
H Blubber 25 gm ☐ (LN)

Muscle (Describe):

Tissue Set Jar #1
Muscle ☐ (FM)

Sp#_____ A Muscle 1-100gm ☐ (LN)

Collect additional muscle sample if euthanasia:
B Muscle 25 gm ☐ (LN)
C Muscle 25 gm ☐ (LN)
D Muscle 25 gm ☐ (LN)
E Muscle 25 gm ☐ (LN)

Tissue Set Jar #2
Muscle ☐ (FM)

Brachial plexus (Describe):

Prescapular/Axillary/Brachial plexus (nerve junction-line from thymus to shoulder, vessels and nerve tissue may be collected as one). Separate integument from rib cage and take nerve bundle.

Tissue Set Jar #1
Brachial plexus ☐ (FM)

Tissue Set Jar #2
Brachial plexus ☐ (FM)

Thyroid glands (Describe):

Tissue Set Jar #1
Left Thyroid ☐ (FM)

Tissue Set Jar #2
Right Thyroid ☐ (FM)

Open the chest and perform all collections within the chest. Do not open the abdomen yet. Examine and describe external surfaces of lungs and heart.

After first opening the chest, use sterile syringe to extract chest fluid. Chest fluid will be present around the lungs or
pooled along the diaphragm.

Sp #_____ Chest Fluid ☐ (LN)
Thymus (Describe):

Tissue Set Jar #1
Thymus ☐ (FM)

Tissue Set Jar #2
Thymus ☐ (FM)

Heart (Describe):

Attach sterile 18 g x 1.5" needle to sterile syringe and insert needle to collect pericardial fluid if present. Use same procedure with new sterile needle set up for whole blood collection from the heart.

Sp #_____ Pericardial Fluid ☐ (LN)

Sp #_____ Whole Blood ☐ (LN)

Tissue Set Jar #1

Right Atrium	☐ (FM)
Left Atrium	☐ (FM)
Right Ventricle	☐ (FM)
Left Ventricle	☐ (FM)
Intraventricular Septum	☐ (FM)
Pulmonary Arteries	☐ (FM)
Aorta	☐ (FM)

Tissue Set Jar #2

Right Atrium	☐ (FM)
Left Atrium	☐ (FM)
Right Ventricle	☐ (FM)
Left Ventricle	☐ (FM)
Intraventricular Septum	☐ (FM)
Pulmonary Arteries	☐ (FM)
Aorta	☐ (FM)

Sp #_____ A Heart ☐ (LN)
B Heart ☐ (LN)
C Heart ☐ (LN)

Lungs (Describe):

Tissue Set Jar #1

Trachea ☐ (FM)
Bronchi ☐ (FM)
Right Lung ☐ (FM)
Left Lung ☐ (FM)

Sp #_____ A Lung ☐ (LN)
B Lung ☐ (LN)
C Lung ☐ (LN)

Tissue Set Jar #2

Trachea ☐ (FM)
Bronchi ☐ (FM)
Right Lung ☐ (FM)
Left Lung ☐ (FM)

Mediastinal Lymph Nodes (Describe):

Tissue Set Jar #1

Mediastinal lymph node ☐ (FM)

Tissue Set Jar #2

Mediastinal lymph node ☐ (FM)

Esophagus (Describe any abnormalities):

Tie off both ends of esophagus (from the mouth-pharynx to within 1-2 cm of stomach) and place in bucket with formalin. When tying off the esophagus, first tie off both the distal end of one section and the proximal end of the next section about ½-1 inch apart then make the cut between the ties.

Sp #_____ Esophagus ☐ (FM)

4. ABDOMEN

Liver (Describe):

Collect liver tissues for histopathology (2 sets), toxicology samples (eight, 25 gm samples), biotoxin analysis (four, 25 gm samples), and for microbiology (two lxlxl cm samples). Collect bile from freshly dead seals only.

Tissue Set Jar #1

Liver ☐ (FM)

Tissue Set Jar #2

Liver ☐ (FM)

Sp #_____ A Liver 25 gm ☐ (LN) I Liver 25 gm ☐ (LN) M Liver 1x1x1cm ☐ (LN)

B Liver 25 gm ☐ (LN) J Liver 25 gm ☐ (LN) N Liver 1x1x1cm ☐ (LN)

C Liver 25 gm ☐ (LN) K Liver 25 gm ☐ (LN)
D Liver 25 gm ☐ (LN) L Liver 25 gm ☐ (LN)
E Liver 25 gm ☐ (LN)
F Liver 25 gm ☐ (LN)
G Liver 25 gm ☐ (LN)
H Liver 25 gm ☐ (LN)

Sp #_____ Bile ☐ (LN)

Pancreas (describe):

Tissue Set Jar #1
Pancreas ☐ (FM)

Tissue Set Jar #2
Pancreas ☐ (FM)

Spleen (Describe):

Collect tissues for HP (2 sets) and for microbiology (two lxlxlcm samples) if liquid nitrogen available.

Tissue Set Jar #1
Spleen ☐ (FM)

Tissue Set Jar #2
Spleen ☐ (FM)

Sp# _____ A Spleen C (lxlxl cm) ☐ (LN)
B Spleen D (lxlxl cm) ☐ (LN)

Gastro-intestinal Tract/Alimentary Canal:

Having already tied off the distal end of the esophagus in part 3, examine serosa of tract from ties to anus. When removing GI tract dissect intestines from mesentery, check mesentery for parasites, and surface of intestines for nodules, vesicles, segmented discoloration, or adhesions. Color can be normal, green, congested, purple, black, red, yellow, white, for example; contents can be described as empty, bile, digesta, for example. Unless indicated by severe decomposition or suspect condition, GI tract may be removed in its entirety.

If specimen is too large for a 5-gallon bucket, it can be subdivided as follows:

1. Stomach: ligate (tie-oft) at both ends.
2. Intestine-rectum-anus: ligate at both ends.

For ALL necropsies, measure the length of the various components of the GI tract, including esophagus, stomach, and intestines to rectum. This can be done on fixed tissues later or in the lab. This does not need to be done on fresh tissues in the field unless you have lots of free time.

Measurements:
Mouth (Pharynx) to anus _____ m
Greater curvature of stomach

_____ cm
Lesser curvature of stomach_____ cm

Distal sphincter (end) of stomach to anus _____m
Cecum to anus _____ cm
(Cecum is blind pouch at junction of sm and lg intestine)

GIT (Describe):

Sp #_____GIT ☐ (FM)

Mesenteric Lymph Nodes (Describe):

Tissue Set Jar #1
Mesenteric Lymph Node ☐ (FM)

Tissue Set Jar #2
Mesenteric Lymph Node ☐ (FM)

Peritoneal Fluid (Describe):

Collect approximately 10 ml of peritoneal fluid with sterile syringe and store in cryovial.

Sp #_____Peritoneal Fluid ☐ (LN)

Urogenital System:

Examine and describe kidney, adrenal glands, and reproductive tract.

Kidney (Describe):

Include cortex when collecting kidney. Collect tissues for HP (2 sets), eight, 25 gm samples for toxicology, and two lxlxl cm samples for microbiology if liquid nitrogen available.

Tissue Set Jar #1
Left Kidney ☐ (FM)

Tissue Set Jar #2
Right Kidney ☐ (FM)

Sp #_____ A Kidney 25 gm ☐ (LN)
B Kidney 25 gm ☐ (LN)
C Kidney 25 gm ☐ (LN)
D Kidney 25 gm ☐ (LN)
E Kidney 25 gm ☐ (LN)
F Kidney 25 gm ☐ (LN)
G Kidney 25 gm ☐ (LN)
H Kidney 25 gm ☐ (LN)
I Kidney 1x1x1cm ☐ (LN)
J Kidney 1x1x1cm ☐ (LN)

Adrenal (Describe):

Tissue Set Jar #1

Left Adrenal ☐ (FM)

Right Adrenal ☐ (FM)

Tissue Set Jar #2
Urinary bladder (Describe):

The urinary bladder will normally be found attached to the ventral wall of the abdomen. The urinary bladder can be empty, dilated, or thickened. Examine for tumors.

Attach a sterile 18 g x 1.5" needle to a sterile syringe, insert the needle into bladder, and draw on syringe to collect urine.

Urine: amount: _____ cc color: _____ consistency:_____

Sp #_____ Urine ☐ (LN)

Tissue Set Jar #1
Urinary Bladder ☐ (FM)

Tissue Set Jar #2
Urinary Bladder ☐ (FM)

Reproductive Tract:
MALES (For all males, examine, describe, and sample reproductive tract):

If both testes appear grossly normal, collect a 1 cm thick cross section of both testes for each HP jar and a 1 cm thick cross section of both testes for toxicology.

Tissue Set Jar #1
Left Testes ☐ (FM)
Right Testes ☐ (FM)

Tissue Set Jar #2
Left Testes ☐ (FM)
Right Testes ☐ (FM)

Sp #_____ A Left Testes ☐ (LN)
B Right Testes ☐ (LN)

FEMALES (Externally examine the vestibule and surrounding area and describe observations):

If lactating, collect as much milk as possible and freeze. Collect double HP specimens from each of the four teats and fix in formalin.

Milk (Describe):

Sp _____ Milk ☐ (LN)

Mammary Glands (Describe):

Tissue Set Jar #1

Mammary Gland 1 ☐ (FM)
Mammary Gland 2 ☐ (FM)
Mammary Gland 3 ☐ (FM)
Mammary Gland 4 ☐ (FM)

Tissue Set Jar #2

Mammary Gland 1 ☐ (FM)
Mammary Gland 2 ☐ (FM)
Mammary Gland 3 ☐ (FM)
Mammary Gland 4 ☐ (FM)

Reproductive Tract:
Collect whole **reproductive tracts** from all females, including immature seals and pups. **Include the external genitalia, ovaries, uterus and cervix; measure ovaries; and fix in formalin.** Do not slice open the ovaries (they are small enough that they will fix completely), but note any discolorations, ruptured follicles, etc. Cut several cuts
into the body of the uterus to allow formalin to flow into the lumen for better fixation of endometrium. Note presence of fetus, abscesses, tumors, unusual amounts of fluid, etc. Examine ovaries for symmetry, scars, cysts, or other structures "normal" or "abnormal." Measure ovarian structures.

Sp #_____Female Reproductive Tract ☐ (FM)

Ovaries (Describe):

Measurements: Left Ovary_____cm x _____cm
Right Ovary _____cm x _____cm

For pregnant females, aborted fetuses, or perinatal pup deaths, examine and sample (or collect) umbilicus, placenta and fetus.

Umbilicus (Describe):

Tissue Set Jar #1
Umbilicus ☐ (FM)

Tissue Set Jar #2
Umbilicus ☐ (FM)

Placenta (Describe):

Handle tissues in a sterile manner. Fix samples for histopathology as follows: remove four 5 cm x 1cm **full thickness** strips (extending through to include both the fetal and maternal side) representative of normal and any abnormal portions of the placenta. If freezing is an option, in addition to preserving sections for histopathology, freeze four 5 cm x 1 cm full thickness strips (extending through to include both maternal side) representative of normal and any abnormal portions of the placenta. **If freezing in liquid nitrogen, BE SURE samples are small enough to be removed from the dewar.** If this is not possible, but immediate transportation is available, send to Honolulu on ice. DO NOT STORE TISSUES WITH FOOD FOR HUMAN CONSUMPTION!

Tissue Set Jar #1

Placenta ☐ (FM)
Placenta ☐ (FM)

Tissue Set Jar #2

Placenta ☐ (FM)
Placenta ☐ (FM)

Sp # _____ A Placenta ☐ (LN)
B Placenta ☐ (LN)
C Placenta ☐ (LN)
D Placenta ☐ (LN)

Fetus (Describe):

If fetus is <25 cm in length, split it in half from chin to pubis and place both halves in formalin. If fetus is >25 cm in length, perform complete necropsy.

If complete necropsy is performed, use separate Necropsy Report Form. Handle tissues in a sterile manner.

Take one swab from both the throat and rectum before beginning necropsy.

If fetus is fresh, collect several cc's of fluid from the stomach and freeze in liquid nitrogen.
Ventral Length: _____ cm

Axillary Girth: _____ cm
Mass: _____ kg
Sex: M or F
Condition (Describe):

_____

Fetus Necropsied: Y or N (if Y, see Necropsy # _____)

If Fetus is < 25 cm:
Sp #_____ A Fetus ☐ (FM)
B Fetus ☐ (FM)

OR

If Fetus is > 25 cm:
Sp #_____ Throat Swab ☐ (LN)
Sp #_____ Rectal Swab ☐ (LN)
Sp #_____ Stomach Fluid ☐ (LN)

NECROPSY SPECIMEN REFERENCE TABLE (Listed by order of collection):

Tissue	Diagnostic Activity	Size of Sample	No. Samples	Preservation	Notes
Eye (right), with eyelid and lacrimal duct	Histopathology	Whole eye	1	Make a 2-3 cm cut in the glove along the interface of the sclera (white part) and the part normally in the eye socket	Fix both eyes in FM if freezing not possible
Eye (left), with eyelid and lacrimal duct	PCR/Microscopy	Whole eye	1	Whirlpak in liquid nitrogen	
Aqueous humour	PCR/Micro	As much as possible	Both eyes	Cryovial, in liquid nitrogen	Collect fluid with sterile needle/ syringe
Brain, left half	Histopathology	Slice into 1 cm wide sections	1	10% formalin	Slice from side to side (medial to lateral)
Brain, right half	Histopathology	Slice into 3 sections	1	10% formalin	Slice from front to back
Brain	Toxicology	25 g	8	Wrap in foil, shiny side out, in liquid nitrogen	
Skull	Aging	Whole	1	Dry	Flense outdoors
Mandible	Aging	Lower jaw	1	Dry	Flense outdoors
Nasal swab	Virology		1	Cryovial, in liquid nitrogen	
Nasal mites	Parasitology		1	AG or 10% formalin	If nasal mites present, collect with swab
Genital swab	Virology		1	Cryovial, in liquid nitrogen	
Blubber Biopsy	Fatty Acid Analysis	6 mm	1	5 ml cryovial, in liquid nitrogen	See blubber biopsy protocol
Blubber Biopsy	Toxicology	6 mm	3-4 plugs	Teflon container, in liquid nitrogen	See blubber biopsy protocol
Vibrissae	Histopathology	5 cm X 5 cm X 1 cm	2	Include 2x 2 x 1 cm thick tissue attached to vibrissae	
Flipper, front, w/ nail	Histopathology		2	10% formalin	
Flipper, rear	Histopathology		2	10% formalin	
Flipper, rear	Genetics		1	95% ethyl alcohol	
Lymph nodes, submandibular with salivary gland	Histopathology	3 cm X 3 cm X 1 cm	2	10% formalin	

Tissue	Diagnostic Activity	Size of Sample	No. Samples	Preservation	Notes
Tonsil	Histopathology	5 cm X 5 cm X 1 cm	2	10% formalin	
Tongue	Histopathology	5 cm X 5 cm X 1 cm	2	10% formalin	
Skin	Histopathology	5 cm X 5 cm X 1 cm	2	10% formalin	
Blubber	Histopathology	1 cm X 3 cm X 3 cm	2	10% formalin	Sample includes skin, sub-cutaneous tissue and muscle
Blubber	Toxicology	25 gm	8	Wrap in foil, shiny side out, in liquid nitrogen	Sample from sternal region, without skin
Muscle	Histopathology	5 cm X 5 cm X 1 cm	2	10% formalin	
Muscle, skeletal	Biotoxins	1-100 g	1	Whirlpak, in liquid nitrogen	
Muscle, skeletal	Toxicology	25 gm	4	Wrap in foil, shiny side out, in liquid nitrogen	Collect if euthanasia only
Brachial plexus	Histopathology	All one side	2	10% formalin	
Thyroid	Histopathology	5 cm X 5 cm X 1 cm	2	10% formalin	
Chest Fluid	Pathology	10 ml	1	Cryovial, in liquid nitrogen	
Thymus	Histopathology	5 cm X 5 cm X 1 cm	2	10% formalin	
Pericardial fluid	Pathology	As much as possible	1	Cryovial, in liquid nitrogen	
Whole blood	Biotoxin	1-100 ml	1	Teflon container, in liquid nitrogen	
Heart, aorta	Histopathology	3 cm section	2	10% formalin	
Heart, intraventricular septum	Histopathology	3 cm X 3 cm X 1 cm	2	10% formalin	
Heart, right atrium	Histopathology	3 cm X 3 cm X 1 cm	2	10% formalin	
Heart, left atrium	Histopathology	3 cm X 3 cm X 1 cm	2	10% formalin	
Heart, left ventricle	Histopathology	3 cm X 3 cm X 1 cm	2	10% formalin	
Pulmonary arteries	Histopathology	3 cm X 3 cm X 1 cm	2	10% formalin	

Tissue	Diagnostic Activity	Size of Sample	No. Samples	Preservation	Notes
Heart	Biotoxins	1-100 g	3	Whirlpak in liquid nitrogen	
Trachea	Histopathology	5cm X 5 cm X 1 cm	2	10% formalin	
Bronchi	Histopathology	3 cm long section	2	10% formalin	
Lung, right	Histopathology	5 x 5 x 2.5 cm	2	10% formalin	
Lung, left	Histopathology	5 x 5 x 2.5 cm	2	10% formalin	
Lung	PCR/Micro	1 x 1 x 1 cm	3	Whirlpak in liquid nitrogen	
Lymph nodes, mediastinal	Histopathology	3 cm X 3 cm X 1 cm	2	10% formalin	
Esophagus	Histopathology		1	10% formalin	
Liver	Histopathology	5 cm X 5 cm X 1 cm	2	10% formalin	
Liver	Toxicology	25 g	8	Wrap in foil, shiny side out, in liquid nitrogen	Slice from caudal end of left anterior lobe; collect 8 samples, 12 if euthanasia;
Liver	Biotoxins	25 g	4	Whirlpak in liquid nitrogen	
Liver	PCR/Micro	1x1x1 cm	2	Whirlpak in liquid nitrogen	
Bile	Toxicology	No more than 12 ml	1	Cryovial, in liquid nitrogen	Fresh dead only (codes 1,2)
Pancreas	Histopathology	5 cm X 5 cm X 1 cm	2	10% formalin	
Spleen	Histopathology	5 cm X 5 cm X 1 cm	2	10% formalin	
Spleen	PCR/Micro	1x1x1 cm	2	Whirlpak in liquid nitrogen	
GIT	Histopathology	Whole GIT	1	10% formalin	
Lymph nodes, mesenteric	Histopathology	3 cm X 3 cm X 1 cm	2	10% formalin	
Peritoneal fluid	Microbiology	No more than 12 ml	1	Cryovial, in liquid nitrogen	
Kidney	Histopathology	5 cm X 5 cm X 1 cm	2	10% formalin	Include cortex
Kidney	Toxicology	25 g	8	Wrap in foil, shiny side out, in liquid nitrogen	Take sample from caudal end of each kidney; include cortex

Tissue	Diagnostic Activity	Size of Sample	No. Samples	Preservation	Notes
Kidney	PCR/Micro	1x1x1 cm	2	Whirlpak in liquid nitrogen	
Adrenal	Histopathology	Both	2	10% formalin	
Urine	Toxicology	No more than 12 ml	1	Cryovial, in liquid nitrogen	
Urinary bladder	Histopathology	5 cm X 5 cm X 1 cm	2	10% formalin	
Testes	Histopathology	1cm thick cross section of both	2	10% formalin	
Testes	Toxicology	1cm thick cross section of both	2	Wrap in foil, shiny side out, in liquid nitrogen	
Reproductive tract, female	Histopathology	Entire tract whole	1	10% formalin	
Milk	Nutritional analysis	As much as possible	1	Cryovial, in liquid nitrogen	
Mammary glands	Histopathology	5 cm X 5 cm X 1 cm	8	10% formalin	2 sets of 1 each
Umbilicus	Histopathology	5 cm X 5 cm X 1 cm	2	10% formalin	
Placenta	Histopathology	5 cm long x 1 cm wide	4	10% formalin	Include both fetal and maternal sides
Placenta	Microbiology	5 cm long x 1 cm wide	4	Whirlpak in liquid nitrogen	Include both fetal and maternal sides
Fetus	See notes	See notes	See notes	See notes	Complete necropsy or split in 2 and preserve in formalin
Fetal Throat Swab	Bacteriology		1	Cryovial, in liquid nitrogen	
Fetal Rectal Swab	Bacteriology		1	Cryovial, in liquid nitrogen	
Fetal Stomach Fluid	Microbiology		Several ccs	Cryovial, in liquid nitrogen	
Lesions	Cytology	Slides	2	1 fixed in 95% ethyl alcohol, 1 air dried	Take 2 smears from any lesion
Lesions	Histology	Include the margin between the abnormal and adjacent normal tissue	2	10% Formalin	

APPENDIX C:

Equipment and Supplies for a Single Necropsy

28 Jan2003/RCB

EQUIPMENT AND SUPPLIES FOR A SINGLE NECROPSY

1. Clipboard, metal
2. Necropsy Table (Checklist)
3. Necropsy Report Form(s)
4. Necropsy Manual
5. Scarcard
6. Census Form
7. Tag/handling card
8. Survival factor form
9. Self-sticking pre-printed specimen labels (3 sheets)
10. Waterproof labels ("rite-in-rain" paper, pre-cut) (3 sheets)
11. Pencils (2)
12. Ball-point pens (optional) (2)
13. Permanent markers (sharpies) (2)
14. Ruler (metric)
15. Metal tape measure (metric)
16. Tape measure, flexible (metric)
17. Surgical scissors (2: straight and curved)
18. Rubber gloves
    chemical resistant - 2 pr. latex
    heavy duty - 6 pr. latex exam gloves - 15 pr
19. Scalpel handles (4: 2 #8, 2 #6)
20. Scalpel blades (30)
21. Blubber biopsy punch, 6 mm (2)
22. Forceps (2 pr.)
23. Hemostats (1)
24. Dacron Swabs *(5)*
25. Microscope slides
26. Slide holder for microscope slides
27. Small vials (—2 x ½") - 6 ea.
28. Med. vials (—3' x 2) - 6 ca.
29. Whirlpak bags (20 ea.)
30. Ziploc bags (large)
31. Paper towels
32. Antibacterial soap/Hand sanitizer
33. Sharps container
34. Needles, 1 ½', 18g *(5)*
35. Syringes, 20cc or 35cc *(5)*
36. Blood tubes, PT, TT (4)
37. Biohazard bag (2)
38. Camera with colored slide film and/or Digital camera
39. Shovels
40. Jars and buckets partially filled with 10% NB Formalin
    a. 2 large jars for duplicate sets of histopathology tissue samples
    b. 1 bucket for entire female reproductive tract
    c. 1 bucket for esophagus
    d. 1 5gal. bucket for entire gastrointestinal tract from beginning of stomach to anus
    e. 2 jars for eyes
    f. 2 jars for brain
41. Hacksaw
42. Axe
43. Cryovials (20)
44. String to tie off beginning of stomach, etc. (1 roll)
45. Cutting board (not on spc. coil. list)
46. Disposable aprons (3)
47. Bone shears
48. Archival tag labels for boxes
49. Dissecting tray
50. White or light blue plastic sheet for photographic background
51. 85% saline for relaxing parasites
52. 10% NB Formalin
53. Ethyl Alcohol *95%*
54. Isopropyl Alcohol 70%
55. Glycerol
56. Teflon container (3)
57. Aluminum foil (1 roll)
58. Knives
59. Blank notecard

APPENDIX D:

Hawaiian Monk Seal Rescue and Rehabilitation Guidelines

HAWAIIAN MONK SEAL RESCUE AND REHABILITATION GUIDELINES

I. QUARANTINE

A. QUARANTINE DEFINITION AND OBJECTIVES

1. Quarantine refers to isolation or restriction on travel or passage imposed to keep contagious diseases, insect pests, etc. from spreading. Since 1989, NMFS has maintained quarantine policies within the rehabilitation program.

2. Hawaiian monk seals held at Kewalo Research Facility (KRF) must be maintained under strict quarantine at all times to a) minimize transmission of disease from outside sources, b) minimize the spread of disease from these seals to susceptible animals on Oahu, including humans, and c) minimize transmission of disease among the three holding tanks at KRF.

3. All personnel involved in the feeding, handling, and care of these seals must be properly trained in quarantine procedures by an experienced NMFS or the Joint Institute for Marine and Atmospheric Research (JIMAR) staff. (Quarantine procedures should always be posted in the food preparation area.)

B. NMFS QUARANTINE POLICY

1. All equipment used in the quarantine facility, including feeding, handling, and medical supplies **MUST** be **A)** labeled with **"MONK SEAL QUARANTINE"** and its specific use **B)** used exclusively for quarantined seals **C)** kept separate from equipment used for other animals (wild monk seals, dolphins, etc) and **D)** properly disinfected before and after entering the quarantine enclosures as dictated by Dr. Bob Braun.

2. Due to the unknown risk of infection from these seals, feeding, handling, and medical procedures involving non-quarantined animals should precede contact with the KRF seals whenever possible.

3. Avoid direct contact with domestic or other captive or wild animals before and after entering quarantine enclosure.

4. Shower and change clothes before and/or after going to another animal care facility if entering the KRF seal enclosures on the same day.

5. Wear rubber boots designated for monk seal quarantine use at KRF in the enclosures at all times. Minimize wearing quarantined boots around KRF premises. Separate quarantined boots are provided for tank 3. No street shoes are to be worn into the enclosures, including the walkway leading to tanks 1 and 2.

6. Dip soles of boots in disinfectant footbaths bath upon entering AND leaving all the enclosures.

7. Immediately upon entering the enclosure to tanks 1 and 2, wash hands with antibacterial soap. Also wash hands before and after fish preparation, feeding, or handling seals. Always wash hands immediately after leaving a separate tank enclosure. Before and after entering tank 3, wash hands at the yellow sink next to the enclosure.

8. Any person who will potentially come into direct contact with quarantined seals for any procedure must wear sanitary protective clothing (i.e., coveralls) designated for quarantine monk seal use only. This clothing should be kept clean and in a designated area (in the monk seal lab) away from potential sources of contamination.

9. Restrainers and medical staff must shower after animal handling procedures. Protective clothing worn during procedures should be immediately washed in the KRF washer with soap and dilute bleach solution. Do not set this clothing on any surface outside of quarantine enclosures prior to washing.

II. OBSERVATIONS AND CONDUCT AROUND SEALS

A. INITIAL INSPECTION OF SEALS

1. Prior to feeding, conduct a brief inspection of the seals and tank enclosures.

2. Keys to seal enclosures are kept inside first shelf in MMRP cage (combination #0723).

3. Try to avoid alerting the seals to your presence. Observe condition of seals, water levels, feces (scat), urine, regurgitation (spew), and harmful debris on haul-out areas and on bottom of tanks. When possible, note ID of seal that produced scat, etc.

4. Note anything unusual in a seal's normal appearance (eyes, nasal discharge, bite wounds, etc.) and behavior (lethargic, unresponsive, etc.). Notify attending veterinarian and head caretaker immediately of any abnormal changes in a seal's health.

5. Succinctly record any observations on health record forms (refer to "Instructions for Completing the Hawaiian Monk Seal Feeding and Health Record Form").

B. CONDUCT AROUND SEALS AT ALL TIMES

1. When in enclosures, **DO NOT MAKE PHYSICAL CONTACT WITH SEALS** unless necessary for procedures requiring handling.

2. If seals are resting or sleeping, do not make loud noises or startling gestures, and move slowly when in close proximity to them to minimize stress.

3. Whenever possible, observers should remain as inconspicuous and unobtrusive as possible to observe seals' normal behaviors in captivity and minimize their stress in captivity.

4. Seal aggression displayed during feedings should be controlled through the application of behavior modification techniques. Specific aggressive and undesirable behaviors that are subject to behavior modification include: a) approaching too closely or lunging at the feeder during feedings; b) splashing, sharp movements, open mouth displays, or loud vocalizations which appear as demands for fish; c) approaches toward or contact with the fish bucket; and d) displays of inter-seal aggression. Immediately after the occurrence of any of these behaviors feeders should avoid administering a fish. If the behavior persists or escalates impose a "time out" by temporarily leaving the enclosure with the fish bucket. As a general rule do not administer fish immediately after the occurrence of any undesirable behavior, and attempt to present fish when the seals are orderly, well stationed, and nonaggressive.

5. Outside of feeding sessions seals may display undesirable behaviors which include: a) approaching too closely or too rapidly; b) mouthing hoses, brooms, or boots; and c) stereotypic behaviors which include repetitive splashing or slapping at the walls of the enclosure. If seals approach too closely or too rapidly, use the broom quickly to push the seal away but avoid getting confrontational with seals or allowing the seal to mouth the broom. The mouthing of brooms, hoses, and boots should be discouraged by preventing opportunities for seals to bite at these objects in the first place. Stereotypical behaviors are a sign of boredom and may be reduced by providing seals with their approved environmental enrichment devices (buoys and PVC pipe toys).

III. SEAL CARE

A. FISH PREPARATION

1. Review posted food amounts before preparing fish to note any changes in feeding amounts.

2. Wash hands thoroughly with antibacterial soap before handling buckets and fish.

3. Use buckets hanging in fish house labeled for each tank enclosure. Do not allow unsanitized objects to come in contact with insides of buckets, and minimize such contact with their bottoms. (Dip bottoms of buckets in footbath before feeding if necessary.)

4. Weigh fish from the hanging, digital metric scale. Turn scale "ON," and allow it to zero. Hang bucket, let scale equilibrate, then push the "ZERO" button. After scale has equilibrated to zero again, begin weighing fish. Zero scale for each bucket.

5. Herring is stored in boxes and plastic bags in the freezers marked "MONK SEAL FOOD ONLY." **ALL FISH FOOD MUST HAVE A DOUBLE-LAYER OF PROTECTION AND BE WELL-SEALED TO PREVENT FREEZER BURN AND DRYING.** Always tie plastic bags of herring after weighing fish.

6. **FREEZERS AND REFRIGERATORS MUST REMAIN CLEAN AND NEAT AT ALL TIMES**. All feeders are responsible for maintaining cleanliness of the food preparation area on a daily basis.

7. While weighing fish, inspect and remove any damaged fish (e.g., no head, bruised). Store these fish in a plastic bag in freezer for use by MTRP. Do not place any trash with damaged fish.

8. Thaw fish in refrigerator.

9. As fish are thawing, inspect again for bruises, cuts, or other abnormalities not detectable when frozen. Replace any unacceptable fish.

10. Promptly feed first tank while fish are cold. Place any other thawed fish in refrigerator until ready to feed.

11. Prepare vitamins for each seal in the morning (type and amount per the attending veterinarian's directions). Place vitamins in one fish per seal, inserted behind gills and into body cavity. Pull off a pectoral fin or cut off a small portion of the tail to identify this fish and ensure it is ingested. If a seal normally guts fish, insert vitamins into cut tail, one per fish. If morning vitamins are missed, leave a note that they should be given in the evening.

B. FREE FEEDING

1. WASH HANDS WITH ANTIBACTERIAL SOAP IMMEDIATELY PRIOR TO AND AFTER FEEDING.

2. DIP BOOTS IN FOOTBATH UPON ENTERING AND LEAVING SEAL ENCLOSURES. If bottoms of buckets have touched unsanitized areas, dip them in footbath before entering.

3. Maintain a safe distance from seals at all times. If seals approach gate too closely to allow entrance, use a quarantine broom to keep them away; or throw a fish into water before entering enclosure.

4. ALWAYS KEEP DOOR SECURELY CLOSED WHILE IN SEAL ENCLOSURES.

5. Give pilled fish after observing that fish are being eaten whole (third or fourth fish). Closely observe if pills are ingested or sink to bottom. If a seal does not receive morning vitamins, record this on the feeding record and leave a note for the evening feeder. Note if each seal appears satiated or is still hungry after feeding, and if any unusual behavior or clinical symptoms arise.

6. While feeding, keep seals as far away from feeder and from each other as possible.

7. Feed at the same pace without letting one seal steal from another. If this happens, replace stolen fish with fish from stealer. Try not to throw another fish until all seals have completely ingested their fish.

8. Never allow seals to take fish directly from buckets.

9. When finished feeding, set buckets outside of enclosure and thoroughly clean deck. While cleaning deck, observe seals for any regurgitation, urination, defecation, and presence or absence of worms in feces.

10. If any uneaten fish remain in bucket, weigh and store in trash bag in freezer for discard (not for MTRP). Record how much each seal actually ate, estimating weight of additional fish ingested or lost fish when necessary.

C. DAILY CLEANING

1. Clean scat, urine, fish parts, and wind-blown debris from the decks and walls of each enclosure prior to feeding seals. Use the brooms and fresh water hoses that are specifically designated for the cleaning of each seal enclosure.

2. Do not allow seals to mouth or bite brooms or fresh water hoses. If the hose enters the tank remove it immediately. Bolts have been installed on the railings of each enclosure to hang hoses out of the seals' reach during cleaning.

3. Never allow the broom, hose, or any equipment to remain unattended in a seal enclosure. Return all equipment to its storage area (i.e., coil and hang hose, place broom in Nolvasan bath) area after use.

4. When cleaning, take the opportunity to inspect urine for color, and feces for consistency and parasites. Always record feces and urine in the feeding log and make special note of any unusual findings.

5. As an aid to cleaning enclosure surfaces the decks may be flooded with sea water by temporarily closing the outlet valves. If the outlets are closed for cleaning, be sure to re-open them immediately after cleaning is complete and before feeding begins. Avoid feeding the seals when the enclosure is flooded because this will distribute scales and fish debris over the surface of the decks.

6. Always keep the enclosure doors securely bolted because the seals are very adept at exiting the enclosures through a door left ajar.

7. After feeding, thoroughly rinse all fish scales, blood, and debris from the decks and walls of the enclosure. Special care should be taken to clean scales from doors, door handles, and bolts.

8. The buckets may be rinsed in the sea water inflow but do not allow the seals to touch, mouth, or bite at the buckets. Rinse buckets thoroughly in the fish house sink, then scrub them with scouring powder and a scrubby (inside bottom first, then inside walls, then outside). Rinse the buckets well and hang to dry with lip facing down.

9. The sink and any feeding-related equipment (rubber gloves, cutting board, plastic containers, scrubbies, sink screens etc.) should be cleaned with chlorinated scouring powder after each feeding and rinsed well.

10. Clean any fish scales, blood, or debris from the fish house after each feeding. Mop the fish house floor with a 20% bleach solution after the afternoon feeding.

11. The common walkway to enclosures 1 and 2 should be scrubbed and rinsed after each feeding.

12. Footbaths are changed every 2 days or as needed using 6 tablespoons (3 oz.) of Nolvasan per gallon of water or designated measuring cup attached to each bottle.

13. Check seals one last time before leaving. Ensure gate to enclosure is locked, water hose is turned off, and drain valve is at appropriate setting. If water to tank was turned off/on, recheck main source. **NEVER LEAVE IN A HURRY**; always double check that all tasks are completed.

14. Return key to seal enclosure to cage and securely lock. If no one else is on premises, make sure all doors and gates are securely locked.

D. WEEKLY CLEANING

1. The monk seal tanks and enclosures are drained and cleaned once a week. A minimum of three people should conduct the weekly cleaning.

2. At the start of cleaning, change all foot and broom baths to a 20% bleach solution. Thoroughly rinse all Nolvasan solution from the brooms, broom baths, and footbaths to avoid Nolvasan/bleach reactivity and staining.

3. Any personnel who will be responsible for draining the tanks must receive specific training. The tanks drain in approximately 30 minutes and during this time the seals must be monitored to ensure that they remain in their tank as it is lowered. Care must be exercised to assure that a seal is not stranded on the deck of a drained pool.

4. While the tank is draining, scrub the entire upper deck and walls of the enclosure with a 20% bleach solution. Let the bleach stand for 10 minutes, then thoroughly rinse with fresh water. When using bleach solutions always direct the rinse water away from seals because the bleach solution is a skin and eye irritant.

5. Sanitize the fiberglass partitions and place them inside the tank to isolate seals from the portion of the tank being cleaned. Broom the walls and floor of the tank with 20% bleach solution. Allow the solution to stand for 10 minutes and than thoroughly rinse with fresh water. Goggles and masks are available and should be used when cleaning the tank with bleach.

6. Do not clean more than one half of the tank at a time and always avoid seal exposure to the bleach solution. Keep the seals cool by spraying with fresh water and monitor the seals' behavior and status regularly.

7. After the entire tank has been cleaned and rinsed, remove all cleaning equipment and turn on the sea water inflow.

8. Scrub the common walkway to tanks 1 and 2 with a 20% bleach solution.

9. After all the tanks and walkways have been cleaned, thoroughly rinse the bleach solution from the brooms, broom baths, and footbaths. Replace the Nolvasan solution (3 oz/1 gal) to all broom and footbaths.

IV. PROCURING SEAL SUPPLIES

A. HERRING & CAPELIN SUPPLY

1. Each week, assess the amount of herring (in cases) that can be stored in the freezers at KRF.

2. Call "Unicold Corporation" (Phone 836-2931) one day in advance and arrange a time for the pickup of a specific amount of herring (in cases).

3. Drive to "Unicold" (address: 3140 Ualena Street, Honolulu, HI 96819) and pick up the herring

4. Store the herring cases in the freezers at KRF making sure that any old stock is moved to the fish house freezer first. Always store new cases on the bottom of the freezer and old cases on top.

5. After delivering the herring to KRF, turn in the invoice from "Unicold" along with a pinksheet to Administrative Support Assistant Bonnie Oshiro in the Administrative Office. PIFSC has a blanket purchase order (BPA) with "Unicold" so be sure to notate that it is a BPA charge on the pinksheet before submitting it to Bonnie. If the invoice cannot be taken to Bonnie on the day of pick-up, e-mail her a message indicating the date and the amount of herring picked up.

6. Empty herring boxes are folded and thrown in the dumpster.

B. VITAMINS

1. Vendor varies. Refer to attending veterinarian for type and amount.

V. WATER QUALITY

1. Water sampling should occur on the same day (Tuesday) and time (1000, no later than 1430) each week, the day before tank cleaning. Sample between 270^0 and 360^0 clockwise with water inlet at 360^0.

2. Do not open lid to bottle until immediately before collection; do not contaminate inside of lid or bottle.

3. Place bottle countercurrent and dip underwater approximately 1 foot. Move bottle underwater countercurrent for a few feet and make sure it is at "fill" line, replace lid, and remove from water.

4. Label bottle with the following information:

a. Source of sample: Kewalo monk seal tank
b. Collected by: NMFS/initials of collector
c. Date and time (Tuesday mornings)
d. Tests required: Fecal coliform

5. Fill additional water bottle for temperature control only.

6. Record water sample collection on log sheet.

7. Immediately place samples in small cooler with frozen ice pack (mandatory) for transport to the Food Quality Lab, 3375 Koapaka Street, Suite 314. If transport is not immediate, place samples in refrigerator. Samples must be received no later than 1530 on day of sampling (Monday - Thursday only). Sign initials in log book at Food Quality Lab and pick up a sterile sample bottle with label and temperature control bottle. Store sample bottles in cooler and ice pack in freezer until next sampling.

8. Results are FAXED within 2-3 days and mailed within a week. These counts should not exceed 1000 MF/100 ml. If fecal coliform counts exceed 1000 MF/100 ml, results are reported to Robert Dollar by phone; sampling must be repeated within 24 hours. Promptly notify Dr. Bob Braun if counts are above 1000 MF/100 ml.

9. Enter date, time, coliform count, and any pertinent comments in Quattro Pro file H:\REHAB\COLIFORM.WQ1.

DIRECTIONS TO FOOD QUALITY LAB

The Food Quality Lab is located at 3375 Koapaka Street, Suite 314. On Nimitz highway, turn left on Paea Street and drive past the shopping center (Airport Mini Mart). Turn right on Ualena Street. Lonestar should be on your right and a ramp should be on your left. Drive up the ramp to get to the Food Quality Lab.

VI. VETERINARY CARE/EMERGENCY CARE

1. In case of an emergency or suspected illness, refer to the phone list and call Dr. Bob Braun immediately to discuss symptoms or circumstances of emergency or illness. If Dr. Braun does not return the call within 5-10 minutes, contact Rob Dollar.

2. A veterinarian or trained NMFS staff member will perform any needed biomedical sampling. Collect one lavender top tube (LTT; for CBC) and two serum separator tubes (SST; for serum chemistry and serum banking). Fill LTT halfway and immediately invert to mix additive with blood (insure blood is not clotted). Do not invert SST's. Let SST tubes sit at room temperature for at least 15-30 minutes (but not more than 1 hour); then spin tubes in centrifuge for 10 minutes. Draw off serum using pasteur pipet into 2 portions (at least 2 ml to send to CVD and remaining sample to freeze in cryovials for monk seal serum bank). Label tubes with animal ID, date, NMFS, and sample type.

3. Medical supplies for blood sampling, fluid and antibiotic administration, crash kit, and emergency drugs are in the monk seal lab at KRF.

4. All monk seal medications and additional medical supplies are kept at the monk seal lab, KRF.

5. CVD forms, current files for rehab seals, and other rehab-related files are kept in the monk seal trailer at KRF.

OVERVIEW OF ACTIVITIES

1. Conduct initial inspection of seals and enclosure.

2. Weigh out fish and thaw.

3. Turn on water hose outside of enclosure before feeding. Enter enclosure only twice and minimize potential contamination.

4. USE NOLVASAN FOOTBATH BEFORE AND AFTER ENTERING ENCLOSURE.

5. WASH HANDS PRIOR TO AND AFTER FEEDING.

6. KEEP DOOR TO ENCLOSURE SECURELY CLOSED AT ALL TIMES.

7. Feed seals. Note if each seal appears satiated or is still hungry after feeding, and if any unusual behavior or clinical symptoms arise.

8. Clean deck once a day.

9. Wash buckets and other utensils and sanitize sink area. Throw accumulating scales in designated garbage bag in freezer. Properly dispose of any uneaten or damaged fish.

10. Succinctly record appropriate information on feeding forms.

11. Mop floor of fish house.

12. Check seals one last time before leaving. Ensure gate to enclosure is locked, water hose is turned off, and orange drain valve is at appropriate setting. If water to tank was turned off/on, recheck main source. NEVER LEAVE IN A HURRY; always double check that all tasks are completed.

13. Return key to seal enclosure to cage and securely lock cage immediately after initial unlocking to insure keys are not misplaced. If no one else is on premises, make sure all doors and gates are securely locked.

14. If any behavior or other circumstance arises during feeding that is unusual in the recent history of any seal (e.g., lethargy, inappetence, wounds), immediately contact Dr. Bob Braun.

APPENDIX E-1:

Additional Samples Requested During the Laysan 2000 Abortion Investigation

Abortion Investigation, Laysan 2000

Master Sample List (Including UME-specific Requests)

Histology: Samples, preservation, amount and container will be as described in the latest Terry Spraker Field Necropsy Protocol. Addition: Per the request of Terry Spraker two samples 2.5 cm x 5 cm x 5 cm of lung will be put into formalin.

Hematology: Samples, preservation, amount, and container will be as described in the HMS Sample Protocol and performed as in the past epidemiology tier one samples (see Hawaiian monk seal necropsy and specimen collection protocols).

Cytology: Two impression smears each from each lesion will be taken (one air dried and one fixed in 90% ehtono).

PCR:
Live sampling: swab natural orifices (genital, ocular, rectal, oral, and nasal) (sterile swab in cryovial, frozen -20° C)
Necropsy Sampling (2 to 3 sets of tissues): Lymph nodes (mediastinal, mesenteric, and tonsils), liver, lung, spleen, and kidney (1 cm3, in whirlpak bag, frozen -20° C)

Biotoxin:
Live Sampling: feces (plastic bag 1-100 g, frozen -20° C)
Necropsy Samples (1 to 3 sets of samples as possible): Liver, heart, skeletal muscle, feces, stomach contents (1-100 g in whirlpak bag, frozen -20° C); Whole blood and urine (1-100 ml in Teflon container)

Other Toxins: Live Sampling: Whole Blood (5 ml in Teflon or scintillation vials, frozen -20° C), blubber sample (6 mm biopsy punch, amber tox vial or teflon jar, frozen -20° C)

Necropsy Samples (see necropsy protocol)

If Euthanasia: Skeletal muscle, liver (100 g, foil, frozen -20° C)

Fatty Acid: Live sampling: Blubber biopsy (6 mm biopsy punch, 5 ml cryovial, frozen liquid nitrogen)

Bact/Virology:
Live Sampling: Swabs (rectal, genital, ocular, oral, and nasal) (in cryovials frozen in liquid nitrogen), Salmonella rectal swab, aerobic/anaerobic culturette (rectal). One nasal swab (sterile cryovial, in transport media, frozen in liquid nitrogen)

Parasitology: Feces in PVA and GI preserved per Field Necropsy Protocol and Specimen Collection Protocols

Additional: Scats, Spews-Normal protocol. If fresh, frozen in plastic bags (frozen -20° C)

APPENDIX E-2

Specimen Collection Protocols for Specific Biotoxins

Reprinted with permission from: Rowles, T.K., F.M. Van Dolah, and A.A. Hohn. 2001. Gross necropsy and specimen collection protocols. Pp. 449 - 470 In: CRC Handbook of marine mammal medicine (Dierauf, L.A. and F.M.D. Gulland, eds.). 2nd edition. CRC Press, Boca Raton. Copyright CRC Press, Boca Raton, Florida.

TABLE 5 Protocols for Specimen Collection for Biotoxins

Disease	Organism	Toxin	Vector	Tissue/Fluid	Analytical Procedure	Solubility of Toxin	Collection
Paralytic shellfish poisoning (PSP)	*Alexandrium* spp.	Saxitoxin Neosaxitoxin Gonyaitoxin Decarbamoyltoxin	Clams Mussels Zooplankton Fish Water	Stomach contents Liver	MBA RBA ELISA RIA HPLC	Water	Minimum 50 g of tissue or contents into plastic bag or bottle
Amnesic shellfish poisoning (ASP)	*Nitzschia* spp.; *Pseudonitzschia australis*; *Pseudonitzschia* spp.	Domoic acid Isodomoic acid Domoilactones	Mussels Clams Fish Water	Kidney Urine Serum Feces	RBA HPLC MS IP	Water	Minimum 50 g of tissue or contents into plastic bag or bottle; 5–10 ml of serum, whole blood, or urine; brain sections fixed for IP
Neurological shellfish poisoning (NSP)	*Gymnodinium breve*	Brevetoxins	Fish Shellfish Aerosols Water	Respiratory tract Liver Blubber Serum	RBA HPLC IP	Fat	Minimum 50 g of tissue or contents into plastic bag or bottle; 5–10 ml of serum; respiratory or mucosal sections fixed for IP
Ciguatera fish poisoning (CFP)	*Gambierdiscus toxicus*	Ciguatoxins Gambiertoxins	Reef fish (gonads, viscera, liver, flesh)	Liver Kidney	RIA MBA HPLC CT	Fat and water	Minimum 50 g of tissue or contents into plastic bag or bottle
Diarrhetic shellfish poisoning (DSP)	*Donophysis* spp.; *Prorocentrum lima*; *Prorocentrum concavum*	Okadaic acid Donphysistoxin	Clams Mussels	Liver Kidney	CT HPLC ELISA MBA	Fat	Minimum 50 g of tissue or contents into plastic bag or bottle

Key: CT = cellular toxicity; ELISA = enzyme-linked immunosorbent assay; HPLC = high-performance liquid chromatography; IP = immunoperoxidase; MBA = mouse bioassay; MS = mass spectroscopy; RBA = receptor-binding assay; RIA = radioimmunoassay.

APPENDIX E-3

Specimen Collection Protocols for Chemical Pollutants

Reprinted with permission from: Rowles, T.K., F.M. Van Dolah, and A.A. Hohn. 2001. Gross necropsy and specimen collection protocols. Pp. 449 - 470 In: CRC Handbook of marine mammal medicine (Dierauf, L.A. and F.M.D. Gulland, eds.). 2nd edition. CRC Press, Boca Raton. **Copyright CRC Press, Boca Raton, Florida**

TABLE 4 Protocols for Specimen Collection for Chemical Pollutants

Type of Analysis	Specimen	Species	Collection Site	Code	Amount	Storage
Organochlorines	Blubber Liver Brain Blood Other target organs	All	Blubber or cutaneous fat: lateral thorax—full thickness Liver—left caudal lobe	1–2, 3	Minimal 20 g; 100 g optimal for real time; 400 g for archival; >6 ml blood	Frozen in clean glass jars or Teflon jars/bags Minimize contamination of the sample after collection
Polyaromatic hydrocarbons	Bile Liver Blood	Cetaceans	Collect bile from hepatic duct with syringe	1–2 early	50 g tissue 5 ml bile	Frozen (liquid nitrogen) in cleaned container; protect from light; collection should be performed as soon as possible after death; deterioration is rapid
		Pinnipeds Otters Sirenians	Excise gallbladder by clamping off cystic or bile duct; pour bile into container			
Elements	Kidney Liver Skin (epidermis in cetaceans, skin in all others) Blood Target organ	All	Left caudal lobe of liver, left kidney, skin from left lateral wall, whole blood	1–2, 3	A minimum of 20 g	Whirlpak bag or in Teflon bag, freeze at −40°C Collect with stainless steel knife or scalpel

APPENDIX F-1:

Epidemiology Sampling Supply List

Epidemiology sampling supply list

LABORATORY SUPPLIES
glass slides
cover slips
Slide holders
Unopette WBC System (100)
Capillary pipettes for Unopette
critoseal (tube sealer)
Hemacytometer Chamber
Hemacytometer cover glass
Lab counter
Kim wipes
Quickcheck test strips BUN 25/bottle
Glucose test strips/50 strips/vial
Accucheck Advantage Glucometer
glucometer control solution
Disposable transfer pipettes
Non-heparinized HCT tubes
latex gloves
wooden applicator sticks

LABORATORY EQUIPMENT
Clinical Refractometer
Microscope
Spare microscope bulbs
lens paper
lens cleaner
Centrifuge
Microhematocrit centrifuge
light for reading HCT

SWABBING/FECAL COLLECTION
Virology transport media in 1.8 ml cryovials
Virology swabs
Bacteriology swabs -Culturette
C&S bacteriology media (for Salmonella)
PVA fixative solution 32 oz
vials
fecal loops
KYJel
Digital thermometer (rectal)

BIOPSY SUPPLIES
biopsy punches 6 mm
disposable scalpel blades #11
forceps
scissors
xylocaine 2% 50 ml
disposable sterile glove
scintillation vials
amber vials
cryovials, 5.0 ml

CRYOVIALS
1.0 ml
1.8 ml
5.0 ml
nalgene marking pens

SYRINGES
35 cc slip tip
60 cc
12 cc slip tip
6 cc
IV extension sets

NEEDLES
18 gx3.5"
18 gx 1.5"
22gx1.5

VACUTAINER TUBES
SST 9.5 ml
LTT 3 ml
GNTT 5 ml
GNTT 9 ml

DISINFECTANTS, SCRUBBING, ETC
isopropyl
liter Betadine solution
Nolvasan 1 gallon
scrub brush
gauze
distilled water 1 gallon
bleach
paper towels
bottles for scrubbing
soap for handwashing

HANDLING SUPPLIES
tape measure for length and girth
coveralls
masks
hoop net
boots
cotton gloves
knee pads
calipers

FIELD FORMS
necropsy
clinical exam
restraint
lab sheets
specimen collection log
controlled drug log
specimen labels

MISCELLLANEOUS
cooler
Nitrogen Dewar
batteries
pens
pencils
sharps containers
Biohazard bags
latex gloves
Photographic camera w/lens
Film - 400 ASA slides/paper

APPENDIX F-2:

Veterinary Medical Kit

VETERINARY MEDICAL KIT

Ambubag (resuscitation) bag 2.0 L bag
stylet for 10.5" ID endotracheal tube
Lactated Ringer's
0.9% Sodium chloride injection 1 L
Littman Master cardiology stethoscope 24"
Dexamethasone (4mg/ml; 100 cc)
Atropine 1/120 per ml (100 ml)
Diazepam 5 mg/ml vials
Epinephrine 1:1000 20 cc
Soludeltacortef 500 mg; 20 cc
Dopram IV (20mg/ml; 100
8.4% sodium bicarbonate
50% dextrose
Endotracheal tube 14 mm
Endotracheal tube 12 mm
Endotracheal tube 10 mm
IV drip set
hemostats
scalpel handle
sterile scalpel blades
needle holders
absorbable suture
18 gx1.5" catheters
18 gx 5" catheters
3 cc syringes
20 cc syringes
rope

APPENDIX G-1:

Monk Seal Specimen Collection Summary Form

MONK SEAL SPECIMEN COLLECTION SUMMARY: _____ _____

Island Year Page____ of____

Specimen										Preservation Method						Diagnostics						Specimen Storage							
Num	Sub Num	Type	Sub Type	Seal ID	Temp ID	Size	Sex	Survival Factor Num	Collect Date	Islet	Collection	Transport	Arrival	Transport Means	Date Sent	Date Arrived	Lab	Date Sent	Eval.	Result	Date Tested	Facility	Loc.	Shelf	Bin/ Box	Cell	Proj.	Notes	Init.

SPECIMEN TYPE:
BB = blubber biopsy
BC = blood cells
BL = blood
BU = buffy coat
BS = blood smear
ES = eye swab
FL = fecal loop
GI = gastrointest. Tract
MO = molt
NS = nasal swab
NT = necropsy tissue
PC = placenta
PL = the 'pluck'
PS = plasma
RS = rectal swab
RT = reprod. tract
SC = scats
SE = serum
SK = skeletal
SP = spew
ST = stomach contents
TP = tissue plug
VS = vaginal swab
WB = whole blood
WC = whole carcass
WH = whole heart
ON = other necropsy
OS = other swab
OT = other
NO = none (sample discarded)

SUB-TYPE:
AD = adrenal
BC = blubber chunk (necr.)
BM = bact. medium
BP = blubber plug (necr.)
BR = brain
BT = blue top tube
DN = DNA anal.
GI = GI tract
GN = green top tube
GT = grey top tube
KI = kidney
LI = liver
LT = lavendar top
LU = lung
NP = not processed
OL = otolith
PA = parasite
RT = red top tube
SP = spleen
SS = serum sep. tube
VM = viro. medium

PROJECT:
UM = UME
EP = epidemiology
PA = pop. assess.
PS = pup surv
ST = sat. tag.
CC = crit. cam
OT = other

PRESERVATION METHOD:
AA = absolute alcohol (98%)
AF = AFA 50ml 95% ethyl alcohol 5ml acetic acid, 45ml of 10% formalin
AG = 95ml 70% ethyl alcohol, 5ml gly
AL = 70% ethanol
CB = chloroform, BHT mix
DM = DMSO
DR = dry
FR = frozen
FM = 10% buffered formalin
GA = 20% glycerin/80% ethanol
LN = liquid nitrogen
PV = polyvinyl alcohol
RF = refrigerate
RT = room temp.
UF = ultrafreeze
OT = other
NO = none (discarded)

12 Feb 02/TCJ

MONK SEAL SPECIMEN COLLECTION SUMMARY: _____ _____ Page____ of ____
Island Year

Specimen no.	Notes

APPENDIX G-2:

Hawaiian Monk Seal Tagging & Chemical Immobilization Form

22 Jan 1998 / AAA

ATTACHMENT OF INSTRUMENT PLATFORMS TO HAWAIIAN MONK SEALS

Seal ID No_____ Atoll/Island _____ Islet_____ Recorder _____

Date (local) _____ Capture time (local)_____Sex_____Age/Size_____

Axillary girth _____(cm) Dorsal straight length_____(cm)

Release time _____ Release condition _____

Capture time _____ (Start stopwatch)

Time:	Respiration rate:	Heart Rate:	Temperature:	Comments:

Left tag _____ Condition _____ Right tag _____ Condition _____

Left tag _____ Condition _____ Right tag _____ Condition _____

New tag number_____ Side_____ Color_____ Position_____

New tag number_____ Side_____ Color_____ Position_____

Clean injection site THOROUGHLY with betadine and alcohol.

Valium dose (mg) _____ Amount (mL) _____ Valium bottle used _____

Restraint time_____Induction time_____ End time_____Recovery time_____

Total time_____Sedation level (1-4)_____

Blood drawn (Yes / No) Volume _____ Withdrawal time_____

Clean attachment site THOROUGHLY with acetone and sand transmitter just prior to attachment.

Instrument type _____ Instrument number _____ VHF frequency _____

Attachment or retrieval (circle)

Epoxy type_____ Bleach number applied _____

Observations/Comments _____

_____

SUPPLIES AND EQUIPMENT

Emergency kit - seals	Tagging supplies	Needles (1.5 in)	Knife
First aid kit (humans)	Bleaching supplies	Syringes (6 cc min)	Scalpel handles
Endotracheal tube	Knee pads	Valium (w/ extra)	Scalpel blades (large)
Speculum	Sharps container	Acetone	
INSTRUMENT	Trash bag	Clean rags	
Recording form	Alcohol	Cups for mixing epoxy	
Sharpie, pencil	Betadine	Stirring blades	
Watch (w/ stopwatch)	Brush	Gloves	
Hoop net	4 x 4 gauze	Epoxy (w/ extra)	
Water bucket	Needles (3.5 in)	Sandpaper	

APPENDIX G-3:

Hawaiian Monk Seal Clinical Examination Form

NMFS HAWAIIAN MONK SEAL CLINICAL EXAMINATION FORM 23 January 1998/AAA

Seal ID No_____ Sex_____ Age/Size_____ Reason for exam_____

Date_____ Time_____ Location_____ Examined by_____

Weight (kg/lb)_____ Axillary girth (cm)_____ Length (D/V; cm)_____Temperature (rectal; °C)_____

Pre-restraint: Heart rate (resting; b/min)_____ Respiration rate (resting; br/min)_____

Respirations (resting): normal___ labored___ shallow___ congested___ open mouth___ other_____

Nutritional status: emaciated___ thin___ normal___ heavy___ General condition: healthy___ sick___
Explain_____

Behavior: alert___ responsive___ active___ extremely active___ aggressive___ trembling___ lethargic___ unresponsive___
other_____

Vocalizations: y/n___ normal___ abnormal___ describe_____

Mobility: normal___ not using flippers___ (RFF/LFF; RHF/LHF) other_____

External observations: injuries___ scars___ markings___ tags___ (draw and describe on back of form)

Skin/coat: normal___ color_____ molting___ warm___ skin disease___ other_____

Eyes (L/R/B): clear___ tearing___ cloudy___ sunken___ dry___ red___ discharge___ injured___ bleeding___

Nares (L/R/B): dry___ moist___ discharge___ thick/thin/frothy_____ color_____ injured_____

Oral mucous membranes: color_____ moist___ tacky/dry_____ lesions_____ other_____

Feces: y/n___ color_____ consistency_____ volume (ml)____ bloody___ parasites_____

Urine: y/n___ color_____ volume (ml)_____ bloody____ other_____

Sedation: y/n___ drug type_____ dosage (mg/kg)_____ amount (mg)_____ route_____

Restraint: Restraint time (min)_____ Sedation effect_____

LABORATORY TESTS

Samples collected: CBC___ chemistry___ immunology___ heartworm antigen___ serum saved (ml)_____

nasal swab___ rectal swab (microbiology)____ fecal sample (parasitology)____ other_____

Test results (pos/neg): Serology _____

Virology _____ Bacteriology _____ Parasitology _____ Toxicology _____

Salmonella _____ Salmonella serotype(s)_____Other_____

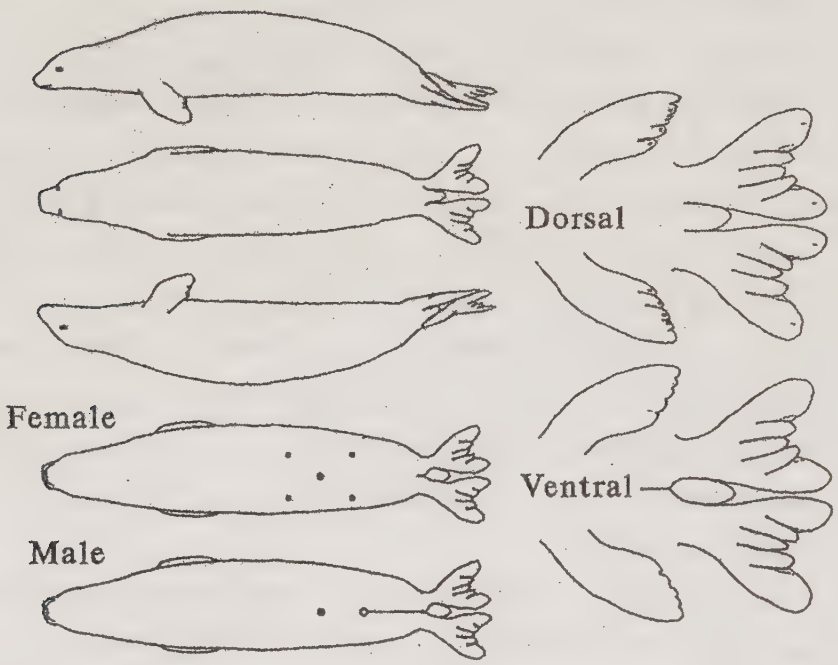
Fecal parasites(y/n)____types_____

Comments_____

External injuries/scars/markings/tags:

Describe_____

Skin abnormalities and markings (include brusies, scratches, wounds, contusions, shark bites, missing flippers, old scars, condition of and tears of skin, external parasites). Attach scar card and make drawings if appropriate.



APPENDIX G-4:

Hawaiian Monk Seal Field Hematology Report

FIELD HEMATOLOGY REPORT

Seal ID No_____ Sex_____ Age/Size_____ Location_____
Blood collection date_____ Blood collection time_____
Volume collected_____ Blood processing time_____

	SST	GNTT	LTT
Hemolysis (none, mild, moderate, severe)			
Lipemia (none, mild, moderate, severe)			
Number of 1.0 mL cryovials			
Number of 1.8 mL cryovials			
Preservation pre-processing			
Preservation post-processing			

Buffy coat: GNTT__ LTT__ No. blood smears: fixed___ unfixed___

WBC: 1_____ 2_____ Ave._____

%PCV: 1_____ 2_____ Ave._____

Total solids:
1_____ 2_____ Ave._____

Glucose:_____ BUN:_____

SWABS			
Salmonella			
Rectal			
Eyes			
Nares			
Oral			
Vaginal			
Prepucial			
Parasite loops			

Blubber depth:

RL___ _____ FL___ _____

RR___ _____ FR___ _____

Blubber biopsy:

RI____ Tox _____

Comments:_____

APPENDIX G-5:

NMFS Human Interaction Forms

MARINE MAMMAL STRANDING REPORT

SID# _____ (NMFS USE)

FIELD NO.: _____ NMFS REGISTRATION NO.: _____

COMMON NAME: _____ GENUS: _____ SPECIES: _____

EXAMINER

Name: _____ Agency: _____ Phone: _____

Address: _____

LOCATION

State: _____ County: _____

City: _____

Locality Details: _____

*Latitude: _____ N

*Longitude: _____ W

TYPE OF OCCURRENCE

Mass Stranding: ☐ Yes ☐ No # Animals _____

Human Interaction: ☐ Yes ☐ No ☐ ?

Check one:
- ☐ 1. Boat Collision
- ☐ 2. Shot
- ☐ 3. Fishery Interaction
- ☐ 4. Other _____

How determined: _____

Other Causes (if known): _____

DATE OF INITIAL OBSERVATION:

Yr. _____ Mo. _____ Day _____

CONDITION: Check one:
- ☐ 1. Alive
- ☐ 2. Fresh dead
- ☐ 3. Moderate decomp.
- ☐ 4. Advanced decomp.
- ☐ 5. Mummified
- ☐ ?. Unknown

DATE OF EXAMINATION:

Yr. _____ Mo. _____ Day _____

CONDITION: Check one:
- ☐ 1. Alive
- ☐ 2. Fresh dead
- ☐ 3. Moderate decomp.
- ☐ 4. Advanced decomp.
- ☐ 5. Mummified
- ☐ ?. Unknown

LIVE ANIMAL — Condition and Disposition:

Check one or more:
- ☐ 1. Released at site
- ☐ 2. Sick
- ☐ 3. Injured
- ☐ 4. Died
- ☐ 5. Euthanized
- ☐ 6. Rehabilitated and released
- ☐ ?. Unknown

Transported to: _____

☐ Died ☐ Released Date: _____

TAGS APPLIED?: ☐ Yes ☐ No

TAGS PRESENT?: ☐ Yes ☐ No

	Dorsal	Left	Right
Tag No.(s):			
Color(s):			
Type:			
Placement		Front/Rear	Front/Rear

CARCASS — Disposition:

Check one:
- ☐ 1. Left at site
- ☐ 2. Buried
- ☐ 3. Towed
- ☐ 4. Sci. collection: (see below)
- ☐ 5. Edu. collection: (see below)
- ☐ 6. Other _____
- ☐ ?. Unknown

NECROPSIED? ☐ Yes ☐ No

MORPHOLOGICAL DATA:

Sex — Check one:
- ☐ 1. Male
- ☐ 2. Female
- ☐ ?. Unknown

Straight Length: _____ ☐ cm ☐ in ☐ est

*Weight _____ ☐ kg ☐ lb ☐ est

PHOTOS TAKEN? ☐ Yes ☐ No

REMARKS: _____

DISPOSITION OF TISSUE/SKELETAL MATERIAL: _____

NOAA Form 89-864
OMB Number: 0648-0178, expires January 31, 2000

Evaluation of Human Interaction

External Examination

A. Body Condition: Emaciated specimens often exhibit sunken epaxial musculature and neck
Emaciated_____ Not Emaciated_____ CBD_____ N/E_____

B. Net or Line Marks: Indicate Y/N/CBD/NE for each area and carefully describe marks:
Head _____ D. Fin_____ L. Flipper_____ R. Flipper_____ Peduncle_____Other_____

_____

_____

C. Fishing Gear Present on Animal (Yes) or (No) Carefully describe:

_____

_____

D. Gear Retained (Yes) or (No)

E. Penetrating Wounds: Yes____ No____ CBD____ N/E____

F. Mutilations: Body slit or otherwise mutilated? Yes____ No____ CBD____ N/E____
Describe:

_____

_____

G. Hemorrhaging/Bruising: Yes____ No____ CBD____ N/E____
Describe extent and area:

_____

_____

Internal Examination

A. Sub-Dermal Hemorrhaging: Yes____ No____ CBD____ N/E____
Describe extent and area:

_____

_____

B. Broken Bones: Yes____ No____ CBD____ N/E____
Describe:

_____

_____

C. Stomach Contents Retained: Yes ____ No____

D. Histopathology Samples Retained: Yes ____ No____

E. Gross Pathology: Yes____ No____ CBD____ N/E____
Describe:

_____

_____

*CBD - Can not be determined
*N/E - Not Examined

APPENDIX G-6:

Marine Forensics Chain of Custody Form (NMFS, SEFSC)

MARINE FORENSICS
Chain of Custody

National Marine Fisheries Service, SEFSC, Charleston Lab
219 Ft. Johnson Rd., Charleston, SC 29412
Phone: (803) 762-8500; FAX: (803) 762-8700

Field reference number:_____

Laboratory reference number:_____

Geographical origin of sample:_____

Name & signature of sample collector:_____

Address of sample collector:_____

_____

Collector's Phone Number:_____ **Date collected:**_____

Seized property# (if applicable):_____

Sample description:_____

_____

THE ABOVE SAMPLE WAS TRANSFERRED AS FOLLOWS:

1. _____ Collector's release signature _____ Method of transfer _____ Date
   _____ Receipt signature _____ Date
2. _____ Release signature _____ Method of transfer _____ Date
   _____ Receipt signature _____ Date
3. _____ Release signature _____ Method of transfer _____ Date
   _____ Receipt signature _____ Date
4. _____ Release signature _____ Method of transfer _____ Date
   _____ Receipt signature _____ Date
5. _____ Release signature _____ Method of transfer _____ Date
   _____ Receipt signature _____ Date
6. _____ Release signature _____ Method of transfer _____ Date
   _____ Receipt signature _____ Date

Each person in possession of the sample must sign and date the form twice, once for receipt of the sample and once for release.

Revised 23 October 1996, WEM.

APPENDIX H-1:

Federal, State and Local Authorities

Name	Title	Office	Address	Phone	E-mail
Jeffrey Polovina	Acting Science Center Director, PIFSC	PIFSC	2570 Dole St, Honolulu, HI 96822	808-983-5390	Jeffrey.Polovina@noaa.gov
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Jason Baker	Leader, Hawaiian Monk Seal Population Assessment	PIFSC	2570 Dole St, Honolulu, HI 96822	808-983-5711	Jason.Baker@noaa.gov
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Samuel Pooley	Acting Regional Director, PIRO	PIRO	1601 Kapiolani Blvd, Honolulu, HI 96814	808-973-2935 x222	Samuel.Pooley@noaa.gov
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Allen Tom	Sanctuary Manager	Hawaiian Islands Humpback Whale NMS	Headquarters Office – Maui, 726 South Kihei Rd, Kihei, HI 96753	800-831-4888	Allen.Tom@noaa.gov
Robert Smith	Reserve Coordinator	NW Hawaiian Islands Coral Reef Ecosystem Reserve Office	308 Kamehameha Ave, Suite 203 Hilo, HI 96720	808-933-8181	Robert.Smith@noaa.gov
Aulani Wilhelm	Assistant Reserve Coordinator	NW Hawaiian Islands Coral Reef Ecosystem Reserve Office	6700 Kalanianaole Hwy, #215, Honolulu, HI 96825	808-397-2657	Aulani.Wilhelm@noaa.gov
Laurie Allen	Acting Director	Office of Protected Resources	1315 East-West Highway, Silver Spring, MD 20910	(301) 713-2319	Laurie.Allen@noaa.gov
Teri Rowles	Marine Mammal Health and Stranding Program	NMFS Office of Protected Resources	1315 East-West Highway, Silver Spring, MD 20910	(301) 713-2322 x178	Teri.Rowles@noaa.gov
Janet Whaley	Marine Mammal Health and Stranding Program	NMFS Office of Protected Resources	1315 East-West Hwy, Silver Spring MD20910	(301) 713-2322 x170	Janet.Whaley@noaa.gov

Name	Title	Office	Address	Phone	E-mail
Gordon Helm	Media Contact	National Marine Fisheries Service		(301) 713-2370 x140	Gordon.J.Helm@noaa.gov
Wende Goo	Management Analyst/ Community Outreach Coordinator	PIFSC	2570 Dole St, Honolulu, HI 96822	808-983-5303	Wende.Goo@noaa.gov
Elton Hout	Office of Ocean and Coastal Resource Management	National Ocean Service	1305 East-West Hwy, Silver Spring, MD 20910	(301) 713-3155 x200	Elton.Hout@noaa.gov
Sylvia Galloway	Center Director, National Centers for Coast Ocean Science	NOAA National Ocean Service	219 Fort Johnson Rd, Charleston, SC 29412	843-762-8525	Sylvia.Galloway@noaa.gov
	Office of Response and Restoration	NOAA National Ocean Service	1305 East-West Hwy, Silver Spring, MD 20910	(301) 713-2989	
Tim Bodeen	Refuge Manager	USFWS Midway Atoll NWR	USFWS Midway Atoll P.O. Box 50167 Honolulu, HI 96850	808-674-8237 x102	tim bodeen@fws.gov
		USFWS Hawaii and Pacific Islands NWR Complex	300 Ala Moana Blvd, Box 50167, Honolulu, HI 96820	808-541-1201	
Capt. Timothy V. Skuby	Commanding Officer	U.S. Coast Guard Marine Safety Office	433 Ala Moana Blvd, Honolulu, HI 96813	808-522-8251	tskuby@d14.uscg.mil
Jeffrey Walters	State Sanctuary Co-manager	State of HI, Dept of Land & Natural Res	1151 Punchbowl St, Rm 330, Hon, HI 96813	808-587-0106	Jeffrey S Walters@exec.state.hi.us
Paul Conry	Wildlife Program Manager	State of Hawaii, DLNR, Division of Forestry and Wildlife	1151 Punchbowl St, Rm 325, Honolulu, HI 96813	808-587-4176	Paul.j.conry@hawaii.gov
Dave Smith	Wildlife Biologist	State of Hawaii, DLNR, Division of Forestry and Wildlife	2135 Makiki Heights Dr, Honolulu, HI 96822	808-973-9786	viking@hgea.org
William S. Devick	Administrator	State of Hawaii, DLNR Div of Aquatic Res	1151 Punchbowl St, Rm 330, Hon, HI 96813	808-587-0100	william.s.devick@hawaii.gov

Information current, November 2003

APPENDIX H-2:

Potential Response Team Members

VETERINARIANS

Name	Address	Phone	Pager/Cell	E-mail
Dr. Bob Braun	44-299 Kaneohe Bay Drive, Kaneohe, HI 96744	808-254-8181	C 808-783-6565	rbraun@lava.net Robert.Braun@noaa.gov
Dr. Les Dalton	SeaWorld of Texas, 10500 SeaWorld Dr., San Antonio, TX 78251	210-523-3278	P 210-746-0232	Les.Dalton@SeaWorld.com
Dr. Chris Dold	The Marine Mammal Center, 1065 Fort Cronkite, Sausalito, CA 94965	415-289-8609	P 415-258-3539	doldc@tmmc.org
Dr. Frances Gulland	The Marine Mammal Center, 1065 Fort Cronkite, Sausalito, CA 94965	415-289-7370	P 415-257-9613	gulland@tmmc.org
Dr. Marty Haulena	The Marine Mammal Center, 1065 Fort Cronkite, Sausalito, CA 94965	415-289-7370	P 415-258-3983	haulenam@tmmc.org
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Dr. John Reif	Colorado State University, Dept. of Environ. Health, Fort Collins, CO 80523	970-491-6074		john.reif@.colostate.edu
Dr. Terry Spraker	Colorado State University, College of Veterinary Medicine, Fort Collins, CO 80523	970-297-4155		tspraker@vth.colostate.edu
Dr. Pam Yochem	Hubbs-SeaWorld Research Institute, 2595 Ingraham St., San Diego, CA 92109	619-226-3874	P 619-242-3551	pyochem@hswri.org

Information current, November 2003

APPENDIX H-3:

Diagnostic Laboratories

	Laboratory	Analysis	Phone	Address
	IDEXX	Serum biochemistry	800-444-4210	2825 KOVR Drive, West Sacramento, CA 95619
Dr. Jeff Stott	U.C. Davis, Laboratory for Marine Mammal Immunology	Virology, immunology	530-752-3349	Dept. of Pathology, Microbiology and Immunology, School of Veterinary Medicine 2054 Haring Hall, 1Shields Ave. Davis, CA 95616
Spencer Jang	U.C. Davis, Veterinary Medical Teaching Hospital	Bacteriology	530-752-1168	VMTH Microbiology Dept./Lab 1011 1 Garrod Drive Davis, CA 95616
Dr. Dori Borjesson	University of Minnesota, Dept. of Diagnostic Medicine	WBC differentials	612-624-7400	Veterinary Diagnostic Laboratory 1333 Gortner Avenue St. Paul, MN 55108
Dr. Klaus Nielson	Animal Disease Research Institute/Canadian Food Inspection Agency	Infectious diseases	613-228-6698	Canadian Food Inspection Agency 3851 Fallowfield Road Nepean, Ontario, Canada K2H8P9
Dr. Ole Nielson	Department of Fisheries and Oceans Canada	Infectious diseases	204-983-5126	501 University Crescent, Winnipeg, Manitoba, Canada R3T 2N6
Dr. Beverly Schmitt	National Veterinary Services Laboratory	Bacteriology	515-663-7266	1800 Dayton Rd, Ames, IA 50010
Dr. Jeremiah Saliki	Oklahoma Animal Disease Diagnostic Lab	Virology	405-744-8809	Farm Road and Ridge Stillwater, OK 74078
Dr. S.K. Hietala	CA Animal Health and Food Safety Laboratory	Bacteriology	530-752-7578	Thurman Lab West Health Services University of CA, Davis Davis, CA 95616
Dr. Tod Leighfield	NMFS Marine Biotoxins Program	Toxicology	843-762-8500	219 Fort Johnson Rd, Charleston, SC 29412
Dr. Gina Ylitalo	Environmental Conservation Division, NFSC	Toxicology	206-860-3325	2725 Montlake Blvd East, Seattle, WA 98112
Dr. Sara Iverson	Department of Biology	Fatty acid analysis	902-494-2566	Dalhousie University, Halifax, Nova Scotia, B3H 4J1, Canada
Dr. Tom Lipscomb	Department of Veterinary Pathology, AFIP	Histopathology	202-782-2600	6825 16th St. NW, Washington, D.C. 20306
Dr. Terry Spraker	Colorado State University	Histopathology	970-419-4155	College of Veterinary Medicine, Fort Collins, CO 80523
Dr. Michael Kliks	CTS Foundation	Parasitology	808-988-7203	3081 G. Paty Dr, Honolulu, HI 96822
Dr. Murray Dailey	The Marine Mammal Center	Parasitology	415-289-7346	1065 Fort Cronkite, Sausalito, CA 94965

Information current, November 2003

APPENDIX H-4:

Cooperating Experts

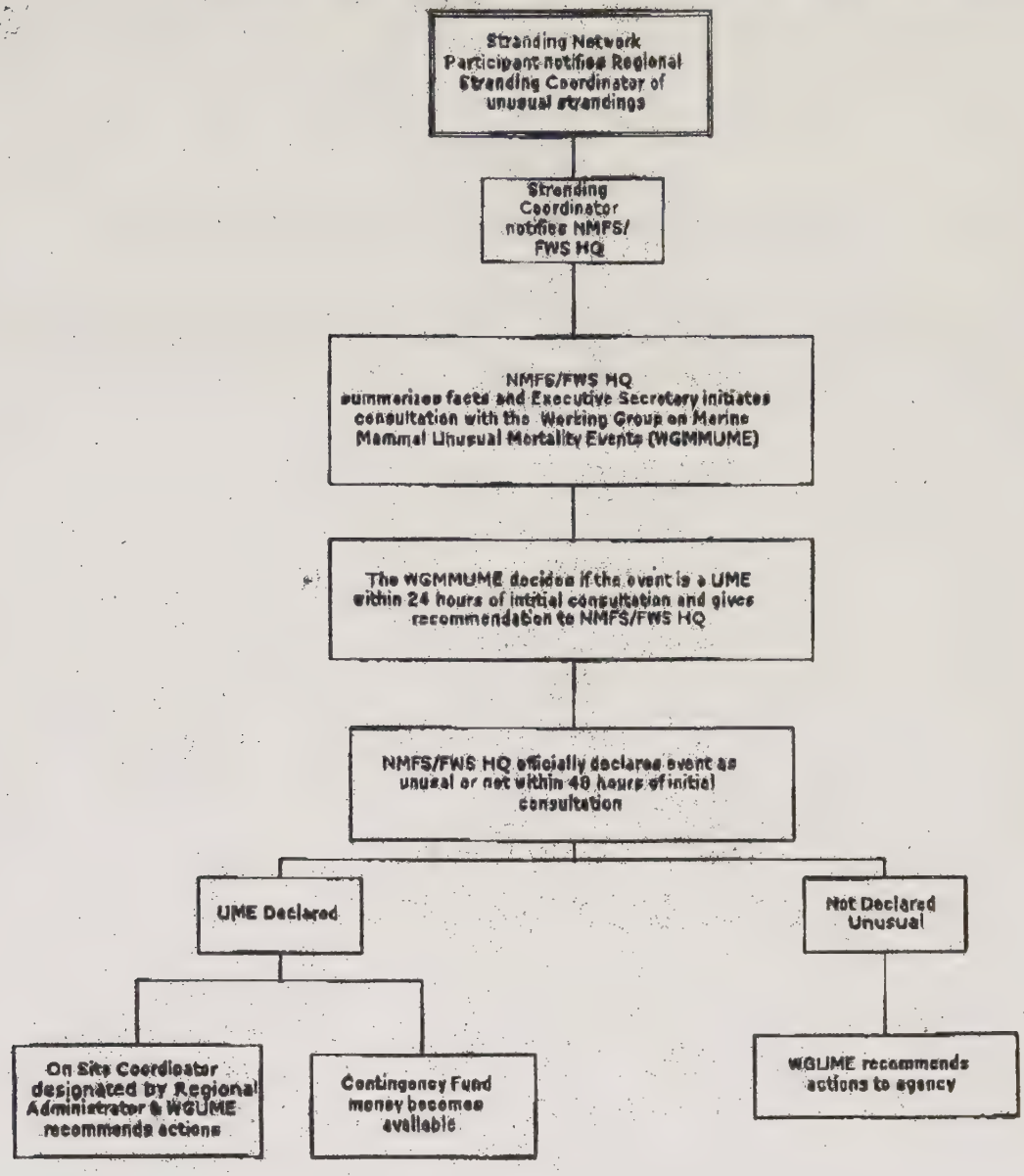
Cooperating Experts			
Name	Address	Phone	E-mail
A. Alonso Aguirre	Wildlife Trust, 61 Route 9W, Palisades, NY 10964-8000	845-365-8368	aguirre@wpti.org
Murray Dailey	The Marine Mammal Center Marin Headlands 1065 Fort Cronkhite Sausalito, CA 94965	415-289-7346 or 415-289-7360	daileym@tmmc.org
William G. Gilmartin	Hawaii Wildlife Fund, P.0. Box 70, Volcano, HI 96785	808-985-7041	bill-gilmartin@hawaii.rr.com
Yoshitsugi Hokama	University of Hawaii at Manoa, Biomed D-105 Honolulu, HI 96822	808-956-5464	hokamay@jabsom.biomed.hawaii.edu
Michael Kliks	CTS Foundation 3081 G. Paty Drive Honolulu, HI 96822	808-988-7203	mmkliks@hawaii.rr.com
Yuanan Lu	Retrovirology Research Laboratory, Pacific Biomedical Research Center University of Hawaii at Manoa Leahi Hospital 3675 Kilauea Avenue Honolulu, HI 96816	808-732-7702	ylu@pbrc.hawaii.edu
James McBain	SeaWorld of California, 500 SeaWorld Dr, San Diego, CA 92109	619-226-3833	james.mcbain@anheuser-busch.com
Ole Nielsen	Central and Arctic Region Fisheries and Oceans Canada 501 University Crescent Winnipeg, Manitoba, Canada R3T 2N6	204-983-5126	
Stephen Rafferty	Animal Health Center 1767 Angus Campbell Road Abbotsford, BC Canada V3G 2M3	604-556-3003	
Timothy Ragen	Marine Mammal Commission 4340 East-West Highway Bethesda, MD 20814	301-504-0087	tragen@mmc.gov

Information current, November 2003

APPENDIX I-1:

Flowchart and Timing of Response to MMUME in the United States

Response Sequence of the WGUME

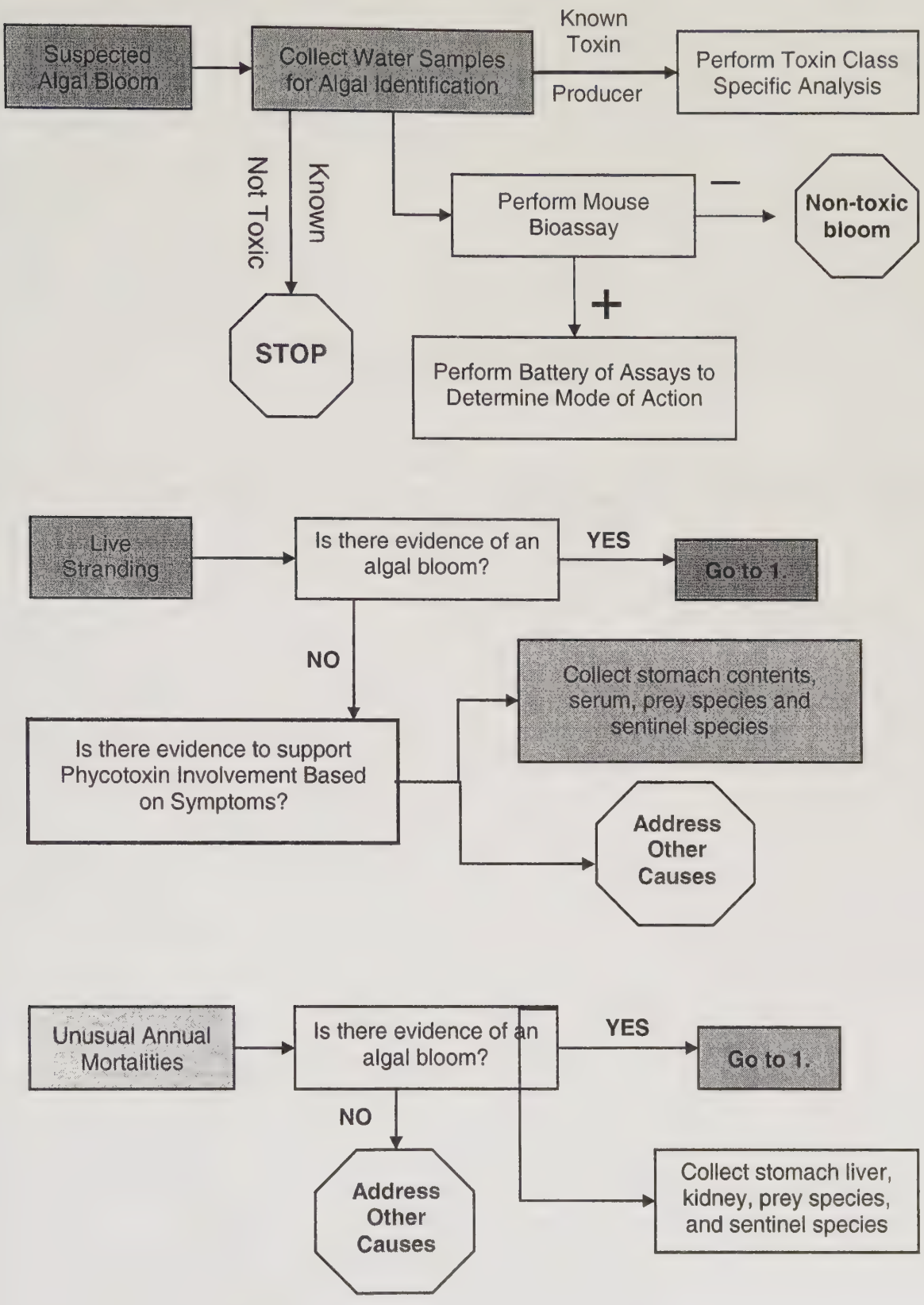

Stranding Network Participant notifies Regional Stranding Coordinator of unusual strandings
Stranding Coordinator notifies NMFS/ FWS HQ
NMFS/FWS HQ summarizes facts and Executive Secretary initiates consultation with the Working Group on Marine Mammal Unusual Mortality Events (WGMMUME)
The WGMMUME decides if the event is a UME within 24 hours of initial consultation and gives recommendation to NMFS/FWS HQ
NMFS/FWS HQ officially declares event as unusal or not within 48 hours of initial consultation
UME Declared
Not Declared Unusual
On Site Coordinator designated by Regional Administrator & WGUME recommends actions
Contingency Fund money becomes available
WGUME recommends actions to agency

APPENDIX I-2:

Flow Diagram for Suspected Marine Biotoxin Incidents

(NOAA Marine Biotoxins Program, http://www.chbr.noaa.gov/CoastalResearch/Pictures/AnalyticalResponseTeam/FlowDiagram.gif)

Flowchart Diagram for Suspected Marine Biotoxin Incidents



APPENDIX I-3:

Flow Chart for Response to a Marine Mammal Mortality Event with Known vs. Unknown Cause

(Wilkinson, D.M. 1996. National Contingency Plan for Response to Unusual Marine Mammal Mortality Events. NOAA Tech. Memo NMFS-OPR-9, 118p. Table 1, between pp. 4 and 5).

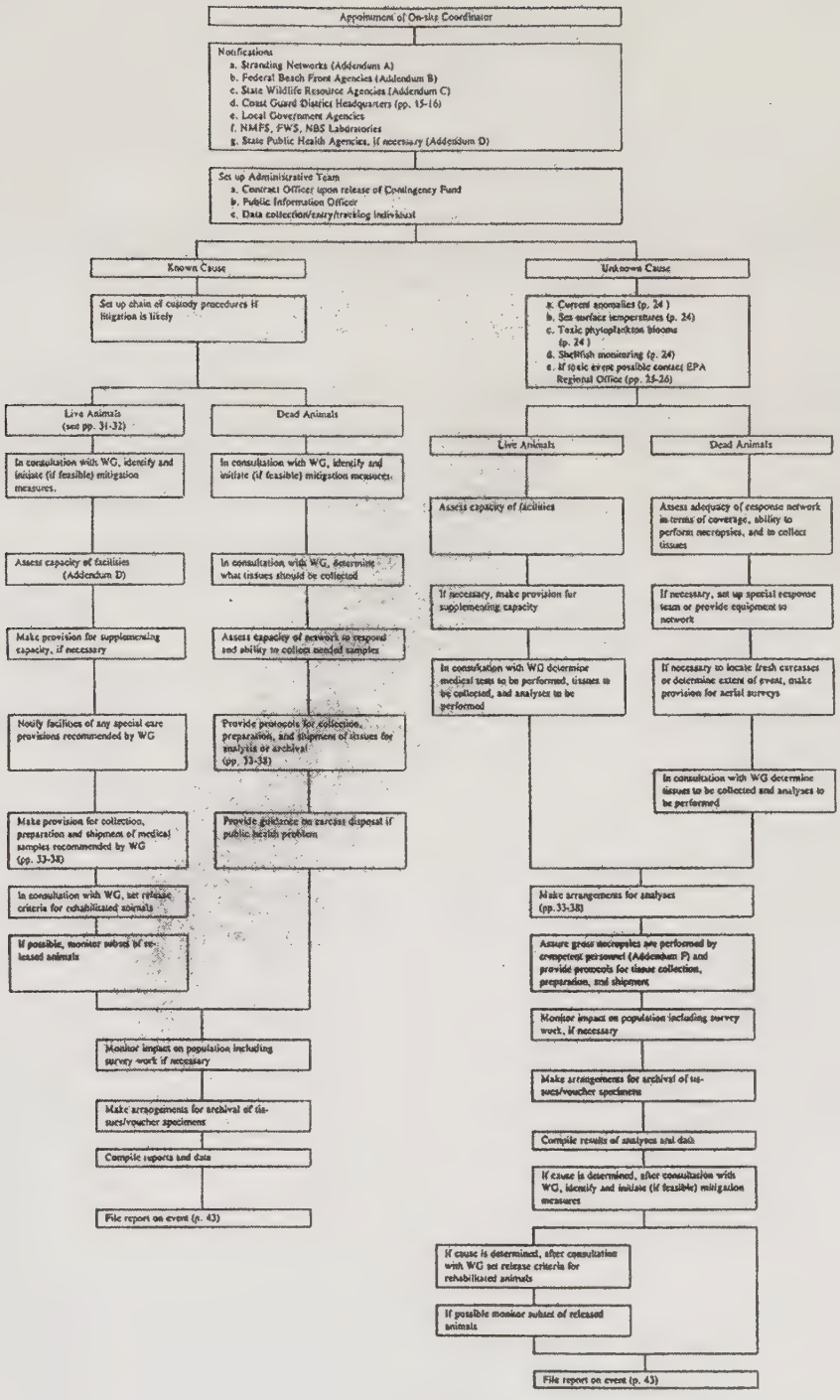

Appointment of On-site Coordinator
Notifications
a. Stranding Networks (Addendum A)
b. Federal Beach Front Agencies (Addendum B)
c. State Wildlife Resource Agencies (Addendum C)
d. Coast Guard District Headquarters (pp. 15-16)
e. Local Government Agencies
f. NMFS, FWS, NBS Laboratories
g. State Public Health Agencies, if necessary (Addendum D)
Set up Administrative Team
a. Contract Officer upon release of Contingency Fund
b. Public Information Officer
c. Data collection/entry/tracking individual
Known Cause
Set up chain of custody procedures if litigation is likely
Live Animals (see pp. 31-32)
Dead Animals
In consultation with WG, identify and initiate (if feasible) mitigation measures.
In consultation with WG, identify and initiate (if feasible) mitigation measures.
Assess capacity of facilities (Addendum D)
In consultation with WG, determine what tissues should be collected
Make provision for supplementing capacity, if necessary
Assess capacity of network to respond and ability to collect needed samples
Notify facilities of any special care provisions recommended by WG
Provide protocols for collection, preparation, and shipment of tissues for analysis or archival (pp. 33-38)
Make provision for collection, preparation and shipment of medical samples recommended by WG (pp. 33-38)
Provide guidance on carcass disposal if public health problem
In consultation with WG, set release criteria for rehabilitated animals
If possible, monitor subset of released animals
Monitor impact on population including survey work if necessary
Make arrangements for archival of tissues/voucher specimens
Compile reports and data
File report on event (p. 43)
Unknown Cause
a. Current anomalies (p. 24)
b. Sea surface temperatures (p. 24)
c. Toxic phytoplankton blooms (p. 24)
d. Shellfish monitoring (p. 24)
e. If toxic event possible contact EPA Regional Office (pp. 25-26)
Live Animals
Dead Animals
Assess capacity of facilities
Assess adequacy of response network in terms of coverage, ability to perform necropsies, and to collect tissues
If necessary, make provision for supplementing capacity
If necessary, set up special response team or provide equipment to network
In consultation with WG determine medical tests to be performed, tissues to be collected, and analyses to be performed
If necessary to locate fresh carcasses or determine extent of event, make provision for aerial surveys
In consultation with WG determine tissues to be collected and analyses to be performed
Make arrangements for analyses (pp. 33-38)
Assure gross necropsies are performed by competent personnel (Addendum P) and provide protocols for tissue collection, preparation, and shipment
Monitor impact on population including survey work, if necessary
Make arrangements for archival of tissues/voucher specimens
Compile results of analyses and data
If cause is determined, after consultation with WG, identify and initiate (if feasible) mitigation measures
If cause is determined, after consultation with WG set release criteria for rehabilitated animals
If possible monitor subset of released animals
File report on event (p. 43)

Table I

APPENDIX J-1:

Memorandum of Understanding with Participants in Unusual Mortality Event Response

(Geraci, J.R. and V.J. Lounsbury. 1997. DRAFT Contingency Plan for Manatee Die-Offs. Prepared for Florida Departement of Environmental Protection. Appendix E.)

Example

APPENDIX E

Florida Department of Environmental Protection

MEMORANDUM OF UNDERSTANDING

with Participants in Manatee Unusual Event Response Activities

---

As a participant in the DEP/FWS response to an unusual event involving manatees, I,

_____, agree to:
(name and institution/organization)

- follow the directives of the Onsite Coordinator(s);
- report all findings and test results to the designated Team Leader or Onsite Coordinator;
- retain tissue samples and/or specimens only by permission from the Federal Onsite Coordinator;
- maintain the security of the investigation; and
- refrain from personal use of data and information gained during the investigation until written authorization is received from the Federal (FWS) Onsite Coordinator.

Signed by:_____ Date:_____
(cooperator)

_____ Date:_____
(FDEP representative)

APPENDIX J-2:

Memorandum of Understanding with Laboratories/Individuals Performing Tests/Analyses Related to Unusual Mortality Event Response Investigation

(Geraci, J.R. and V.J. Lounsbury. 1997. DRAFT Contingency Plan for Manatee Die-Offs. Prepared for Florida Departement of Environmental Protection. Appendix E.)

Example

Florida Department of Environmental Protection

MEMORANDUM OF UNDERSTANDING

with Laboratories/Individuals Performing Tests/Analyses Related to Manatee Unusual Event Response Investigation

---

As a cooperator in the DEP/FWS investigation of an unusual event involving manatees,

_____, agrees to:
(individual and/or laboratory)

- Perform the following tests or analyses:
- _____
  _____
- Process the samples or specimens within the requested time:
  _____Immediately upon receiving.
  _____As soon as possible.
  Within_____.
- Disclose all results and findings.
- Disclose results to only the following person(s):
  _____
  _____
  _____
  (name and position)
- Return samples/specimens (as appropriate) to _____
  _____after use.

The costs for the services described above are:
_____per sample/specimen (or)
_____in total.
Costs shall not exceed_____.
The Florida Department of Environmental Protection agrees to pay theses costs _____.
(payment schedule or final date)

Signed by:_____ Date:_____
(cooperator)

_____ Date:_____
(FDEP representative)

APPENDIX K-1:

Hawaii Area Oil Spill Contingency Plan
Excerpts from 28 August 2001 Update of the Hawaii Area Contingency Plan

(www.uscg.mil/d14/units/msohono/HACP1/index.htm)

U.S. COAST GUARD

Section 1100 - Authority

The Oil Pollution Act of 1990 (OPA 90, Section 4202) amended the Federal Water Pollution Control Act (FWPCA, 33 U.S.C. 1321 (j)). The change directed the development of a National Planning and Response System.

Useful References
Federal Water Pollution Control Act (FWPCA) Title 33 United States Code (USC) Section 1251 et seq.
Oil Pollution Act (OPA) of 1990 Public Law 101-380, August 18, 1990
National Contingency Plan (NCP) Title 40 Code of Federal Regulations (CFR) Part 300
Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) Title 42 United States Code (USC) Section 9601 et seq.

Area Committees

As part of the National Planning and Response System, Area Committees have been established for each area designated by the President. The Area Committees are comprised of qualified personnel from Industry, Federal, State, and local agencies.

The functions of designating areas, appointing Area Committee members, determining the information to be included in Area Contingency Plans, and reviewing and approving Area Contingency Plans has been delegated by Executive Order 12777 (signed October 22, 1991) to the Commandant of the U.S. Coast Guard (through the Secretary of Transportation) for the coastal zone, and to the Administrator of the Environmental Protection Agency for the inland zone.

Each Area Committee is responsible for planning for joint response efforts, including establishing appropriate procedures for mechanical recovery, dispersal, shoreline cleanup, protection of sensitive environmental areas, and protection, rescue, and rehabilitation of fisheries and wildlife. In addition, the Area Committee is required to work with State and local officials to expedite decisions for the use of dispersants and other mitigation substances and devices.

Area Committee Plan

Each Area Committee, under the direction of the Federal On-Scene Coordinator (OSC) for the area, is responsible for developing an Area Contingency Plan (ACP). Which, when implemented in conjunction with the National Contingency Plan (NCP), shall be adequate to remove a worst case discharge of oil or a hazardous substance, and to mitigate or prevent a substantial threat of such a discharge, from a vessel,

offshore facility, or onshore facility operating in or near the geographic area.

Inland and Coastal Zones

The Coast Guard has directed Area Committees to prepare Area Plans based on coastal zone areas in the Captain of the Port (COTP) zones. The Honolulu COTP zone is described in Section 1400 of this plan.

The term "coastal zone" is defined in the current National Contingency Plan (NCP, 40 CFR 300.5) to mean "all United States waters subject to the tide, United States waters of the Great Lakes, specified ports and harbors on inland rivers, and the waters of the contiguous zone, other waters of the high seas subject to the NCP, and the land surface or land substrata, ground waters, and ambient air proximal to those waters."

On an island, with its extensive coastline, it would be unproductive to create detailed maps showing the boundary between the coastal and inland zones. Instead, the following criteria is used to determine if a specific location is within the inland or coastal zone:

Is the source of the spill in or immediately adjacent to waters used for commerce or waters affected by tide?

- If the answer is yes, then it is in the coastal zone.
- If the answer is no, it is in the inland zone.

An example of an "immediately adjacent" area would be a spill that threatens waters defined by these criteria originating from a waterfront facility.

[Back to Section 1000 TOC]
[Back to Index]

U.S. COAST GUARD

Section 1400 - Geographic Boundaries

Area of Responsibility

The Area of Responsibility (AOR) of Marine Safety Office Honolulu (MSO HONO) includes; the Hawaiian Islands, the Territory of American Samoa, Johnston Atoll, Wake Island, Midway Island, Howland Island, Baker Island, Jarvis Island, Palmyra Island, Kingman Reef, and all other territories of the United States, in the Pacific Ocean South/West of a line from 40°N., 150°W. through latitude 5°S., 110°W.; the ocean area west and south of a line running from position 51°N., 158°E. to position 43°N., 165°E.; thence due south to latitude 40°N.; thence due east to longitude 150°W.; thence southeasterly through latitude 5°S., longitude 110°W.

Useful Resources
Coast Guard Regulations Title 33 Code of Federal Regulations (CFR) Sections 3.70-1 and 3.70-10

Not included in MSO Honolulu's AOR is the Territory of Guam, the Commonwealth of the Northern Mariana Islands and Palau, which are in the COTP Guam's AOR.

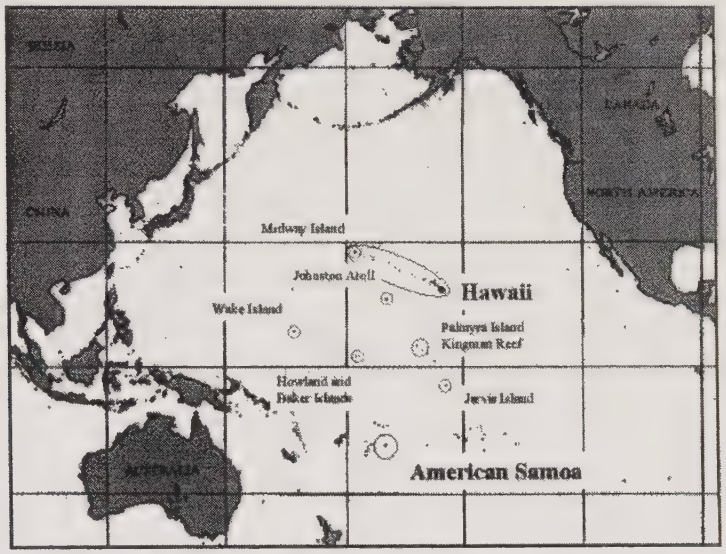


Figure 1400-1, FOSC's Honolulu, Hawai`i Area of Responsibility (AOR)

Area Division

APPENDIX K-2:

Hawaii Area Oil Spill Contingency Plan
Excerpts from Oil Spill Field Operations Guide

(www.uscg.mil/d14/units/msohono/ics/fog/index.htm)

INCIDENT COMMAND SYSTEM

FIELD OPERATIONS GUIDE

ICS-OIL

Table of Contents

0000 - Contents

1000 - Introduction

2000 - Command

3000 - Operations

4000 - Planning

5000 - Logistics

6000 - Finance/Admin

7000 - empty

8000 - empty

9000 - Documentation

==> To Contact Us <==

Field Operations

Guide

ICS-OS-420-1

June 1996

Report All Oil and Chemical Spills

1-800-424-8802

VIEW the Contents Section
How to Acquire a Copy

How to Contact Us

Section 1300 - Common Responsibilities

The following are responsibilities applicable to all ICS personnel:

1. Receive assignment, notification, reporting location, reporting time and travel instructions from your home agency.

2. Upon arrival at the incident, check-in at designated check-in locations. Check-in locations may be found at:

- Incident Command Post.
- Base or Camps, Staging Areas, Helibases,
- Division Supervisors (for direct line assignments).

3. Agency representatives from assisting or cooperating agencies report to Liaison Officer at the Command Post after checking in.

4. All radio communications to Incident Communications Center will be addressed: "(Incident Name) Communications".

5. Use clear text and ICS terminology (no codes) in all radio transmissions.

6. Receive briefing from immediate supervisor.

7. Acquire work materials.

8. Organize, assign and brief subordinates.

9. Complete forms and reports required of the assigned position and send material through supervisor to Documentation Unit.

10. Respond to demobilization orders.

11. Brief subordinates regarding demobilization.

Section 3500 - Wildlife Branch

Wildlife Branch Director

The Wildlife Branch Director is responsible for minimizing wildlife losses during spill responses; coordinating early aerial and ground reconnaissance of the wildlife at the spill site and reporting results to the Situation Unit Leader; employing wildlife hazing measures as authorized in the Incident Action Plan; and recovering and rehabilitating impacted wildlife. A central wildlife processing center should be identified and maintained for: evidence tagging, transportation, veterinary services, treatment and rehabilitation storage and other support needs. The activities of private wildlife care groups, including those employed by the responsible party, will be overseen and coordinated by the Wildlife Branch Director.

a. Review Common Responsibilities (page 3-1).

b. Develop Wildlife Branch portion of the Incident Action Plan.

c. Supervise Wildlife Branch operations.

d. Determine resource needs.

e. Review suggested list of resources to be released and initiate recommendation for release of resources.

f. Assemble and disassemble teams/task forces assigned to the Wildlife Branch.

g. Report information about special activities, events, and occurrences to Operations Section Chief.

h. Maintain Unit/Activity Log (ICS 214).

Section 3510 - Wildlife Recovery Group

Wildlife Recovery Group Supervisor

Under the direction of the Wildlife Branch Director, the Wildlife Recovery Group Supervisor is responsible for coordinating the search for collection and field tagging of dead and live impacted wildlife and transporting them to processing center(s). This group should coordinate with Planning (Situation Unit) in conducting aerial and group surveys of wildlife population in the vicinity of the spill. They should also deploy acoustic and visual wildlife hazing equipment as needed.

a. Review Common Responsibilities (page 3-1).

b. Determine resource needs.

c. Establish and implement protocols for collection and logging of impacted wildlife.

d. Coordinate transportation of wildlife to processing stations(s).

e. Brief the Wildlife Branch Director on activities.

f. Maintain Unit/Activity Log (ICS 214).

Section 3520 - Wildlife Rehabilitation Center

Under the direction of the Wildlife Branch Director, the Wildlife Rehabilitation Center is responsible for receiving oiled wildlife at processing center, recording essential information, collecting necessary samples, and conducting triage, stabilization, treatment, transport and rehabilitation of oiled wildlife. The center is responsible for assuring appropriate transportation to appropriate treatment centers for oiled animals requiring extended care and treatment.

a. Review Common Responsibilities (page 3-1).

b. Determine resource needs and establish processing station for impacted wildlife.

c. Process impacted wildlife and maintain logs.

d. Collect numbers/types/status of impacted wildlife and brief the Wildlife Branch Operations director.

e. Coordinate transport of wildlife to other facility.

f. Coordinate release of recovered wildlife.

g. Implement demobilization plan.

h. Brief the Wildlife Branch Director on activities.

i. Maintain Unit/Activity Log (ICS 214).

Availability of NOAA Technical Memorandum NMFS

Copies of this and other documents in the NOAA Technical Memorandum NMFS series issued by the Pacific Islands Fisheries Science Center are available online at the PIFSC Web site http://www.pifsc.noaa.gov in PDF format. In addition, this series and a wide range of other NOAA documents are available in various formats from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161, U.S.A. [Tel: (703)-605-6000; URL: http://www.ntis.gov]. A fee may be charged.

Recent issues of NOAA Technical Memorandum NMFS-PIFSC are listed below:

NOAA-TM-NMFS-PIFSC-1 The Hawaiian monk seal in the Northwestern Hawaiian Islands, 2001.
T. C. JOHANOS and J. D. BAKER (comps. and eds.)
(April 2004)